THEORETICAL COMPARISON OF SPECTRAL IMAGING METHODS FOR MEASURING ANEUPLOIDY

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1. Introduction

Aneuploidy in cells gives a strong indication of a malignancy and can be determined after Fluorescent In Situ Hybridization (FISH) labeling of the chromosomes inside a nucleus. Counting of the number of copies of each chromosome type in a nucleus will aid in characterization of the cells and may help in diagnosing cancer patients. High throughput analysis of a blood sample containing thousands of cells is a requirement to reach accurate statistics. In metaphase chromosome spreads, the task of counting all 24 different chromosomes was completed a decade ago [1], but this method is relatively slow and sample preparation is a tedious and difficult task. Therefore, we search for the optimal method for fast counting of chromosomal abnormalities in interphase chromosomes. Here we compare three spectral methods using fluorescence widefield imaging.

2. Theoretical comparison of Spectral Methods

The three spectral methods compared are Fourier Interferometer Spectral Imaging, Dispersive Spectral Imaging and Direct Imaging. The Fourier method uses a Sagnac interferometer to create a spectral signature of each pixel at a CCD camera. Dispersive methods employ a grating or prism to disperse the emission light onto the camera, while the Direct Imaging method relies on the parallel recording of several fluorescence channels separated by dichroic mirrors. The labeling procedure will be ratio labeling with 5 or 6 quantum dot probes to create 24 different combinations as was done in a similar way in [1].

Simulation of and experiments with the different methods should yield the method with the highest Signal to Noise Ratio (SNR) of the recorded probes per unit measuring time. This is the most important parameter for high throughput screening. We started with a setup that features the Direct Imaging principle; it contains 2 CCD cameras to image 6 fluorescence channels in parallel.

3. 3D imaging with deconvolution

Overlapping probes give a wrong intensity ratio at the camera and therefore ways were sought to separate the probes correctly. 3D imaging and the help of deconvolution (Huygens, SVI) is implemented in our current setup. The deconvolution step improves the contrast of the images by removing out-of-focus blur. In the deconvolved images, the probes are recognized in a more accurate manner. Future measurements must prove how many different probes can be distinguished and what the minimum measuring time will be. Eventually the spectral imaging method with the highest SNR will be chosen as the future setup for imaging aneuploidy.

[1] M.R. Speicher, S. Gwyn Ballard, D.C. Ward, "Karyotyping human chromosomes by combinatorial multi-fluor FISH", *Nature Genetics*, **12 (4)**, 368-375 (1996).