

COMPARISON OF SPECTRAL IMAGING METHODS BY SIMULATION FOR MEASURING ANEUPLOIDY WITH QDOT LABELED FISH PROBES

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KEY WORDS: Image cytometry, Spectral imaging, Widefield microscopy, FISH, Aneuploidy, Simulation.

1. INTRODUCTION

Aneuploidy in cells is a strong indication for malignancy and can be determined through labeling of the centromeres of the chromosomes by Fluorescent In Situ Hybridization (FISH). Counting of the number of copies of each chromosome type in a nucleus will aid in characterization of the cells and may help in diagnosis of cancer. Our objective is to determine whether or not cells that are identified as tumor cells in blood of patients by immunophenotype are indeed cancerous. The task of counting all 24 different chromosomes was completed a decade ago [1], this was however performed on metaphase spreads which cannot be obtained from the rare circulating tumor cells. Therefore, we search for the optimal method for fast counting of chromosomal abnormalities in interphase nuclei. Here we compare four spectral methods using fluorescence widefield imaging by simulation.

2. THEORETICAL COMPARISON OF SPECTRAL METHODS

The four spectral methods compared are Fourier Interferometer Spectral Imaging, Dispersive Spectral Imaging, Liquid Crystal Tunable Filtering (LCTF) 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. The LCTF scans the whole wavelength range of interest by recording narrow wavelength bands selected by the tunable filter, while in the Direct Imaging method parallel recording of several fluorescence channels -separated by dichroic mirrors- is performed.

A test object containing 24 FISH probes consisting of combinations of Qdots 585, 605, 655, 705 and 800 and the nuclear counter stain DAPI was created on a computer. This combinatorial labeling scheme could in the future be used as complete labeling scheme for labeling all centromeres in target cells. The test object was recorded in a simulation by each spectral method at such integration times that the FISH probes could just be classified correctly by our classification algorithm. All methods were optimized spectrally for imaging the test object. In this way, the fastest spectral method for this experiment could be selected.

3. RESULTS AND CONCLUSIONS

Total imaging times to accurately separate and enumerate the 24 FISH probes per cell recorded for the different spectral methods were 1.05 s for the Fourier method, 0.05 s for the dispersive method, 2.01 s for the LCTF method and 0.01 s for the Direct imaging method. From these simulation results, it can be concluded that the Direct Imaging will be the fastest method for aneuploidy measurements using a scheme with 5 Qdots and DAPI as a counterstain. We will therefore implement this method in the near future.

[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).