

3D SKY: Spectral Karyotyping in the Interphase Nucleus

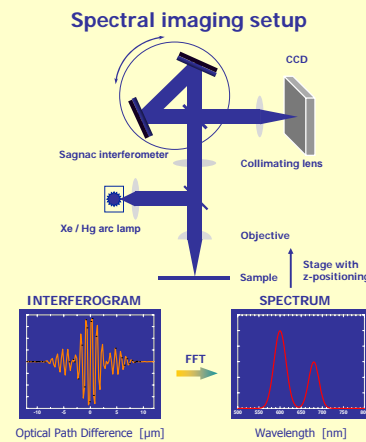
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Introduction

The structure and organization of the genome in the nucleus change along the cell cycle and during cancer progression. One of the more recent structures found in mammalian nuclei is that of chromosomal territories (CT). The chromatin of every chromosome has a distinct and non-overlapping space in the 3D interphase nucleus. We introduce a novel technique to image and classify all CTs simultaneously in the 3D interphase nucleus. The basis of this technique relies on a proven technique known as spectral karyotyping (SKY).

Imaging

The chromosomes are differentially stained with five fluorochromes using combinatorial labeling after which the spectrum is acquired using the following imaging setup.

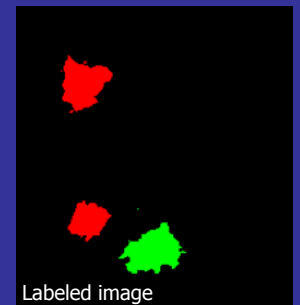
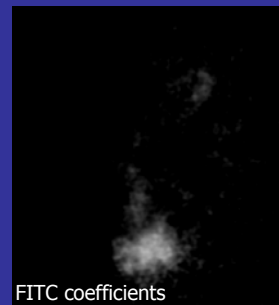
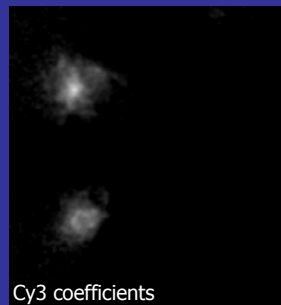
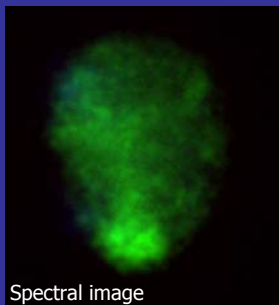


Restoration

A 4D image is made by sequentially shifting the focus plane and acquiring a spectral image. This results in a 4D image: $I(x, y, z, \lambda)$.

Since the imaging set-up is wide-field based, the resulting 3D images at every wavelength contain a high amount of out-of-focus blur. To obtain correct optical sections restoration is performed at every wavelength.

For this the RL-Conchello restoration algorithm is implemented.

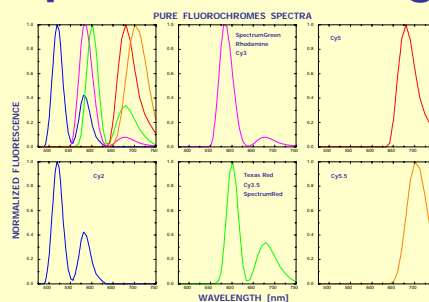


Example

In the insert we see an example of a relatively simple case in which only two chromosomes are stained and only one focus plane is imaged. In the leftmost image we see the nucleus of a mouse lymphocyte as seen through the microscope using a SKY triple band filter. The next two images are the result of spectral unmixing using the two measured spectra from the dyes (Cy3 and FITC).

Given a threshold we can now assign a label to every pixel. In this particular example we see three chromosomal territories, two regions with a certain chromosome and only one region with the other.

Spectral unmixing



The assumption is that the measured spectrum, g , in every voxel is composed of a linear combination of the emission spectra of the dyes used, f_i :

$$g = \sum_{i=1}^5 a_i f_i$$

a_i are the mixing coefficients, the proportions of each dye. Simple matrix inversion algorithms are applied to find these mixing coefficients.

Future

At this point we have implemented the restoration and spectral unmixing algorithms in MatLab.

Our next goal will be to extend the 2D example shown here to 3D. We suspect the biggest hurdle to be:

Fluorochrome bleaching because of the long exposure time during the acquisition of a spectral image.