

3D SPECTRAL KARYOTYPING: DECONVOLUTIONS AND SPECTRAL UNMIXING

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The chromosomal territory (CT) is one of the structures in the mammalian cell nucleus that has become of interest in cell biology. In recent years more and more evidence has arisen that links function with structure within the cell nucleus. To gain more insight into these CTs, we are developing a novel method called three-dimensional spectral karyotyping (3D-SKY). This method is based on a proven method, where chromosomes are combinatorially labeled with five different fluorophores. We use a Sagnac interferometer that enables us to acquire a wide-field spectral image. That is, for a range of lateral positions (x, y) and a fixed axial position $z=z_0$, we acquire $I(x, y, z=z_0, \lambda)$. Sequentially shifting the focal plane, z , and acquiring a series of spectral images gives us a 4D image. After deconvolution and spectral unmixing, we use the combinatorial table to classify each voxel to a specific chromosome or the “background”.

We have studied the influence of deconvolution and spectral unmixing in simulation experiments, specifically the order of processing. See Figure 1 for the simulation model. Intuitively, we first deconvolve and then apply the spectral unmixing algorithm. This means we need to deconvolve n times if we have measured the spectrum at n sampling points (wavelengths). After this we need to spectrally unmix the N dyes.

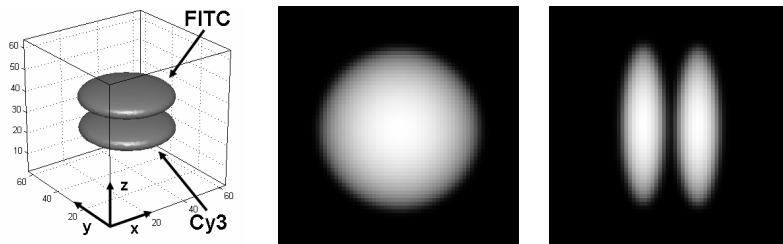


Figure 1: Simulated model of two chromosome domains. (left) in 3D, (center) the (x,y) projection, and (right) the (x,z) projection. Simulated sizes are $64 \times 64 \times 64$ voxels = $6 \mu\text{m} \times 6 \mu\text{m} \times 21 \mu\text{m}$

An alternative approach is to first apply the spectral unmixing and then perform the deconvolutions. With this approach, we spectrally unmix N dyes, after which we only need to deconvolve for the dyes, that is, N times. This second approach is superior because it is less time consuming. Typical values for 3D-SKY are $n \approx 2-5$ and $N \approx 40$.

In this presentation we will show the results of 3D-SKY imaging and the results of our simulations concerning the processing. We anticipate 3D-SKY becoming an important tool in future cell biology research.