# A multi-method driven evaluation of molecular imaging techniques

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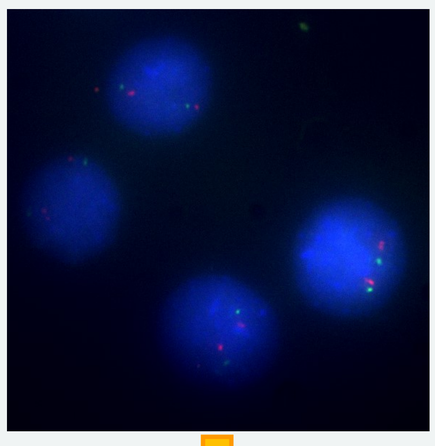
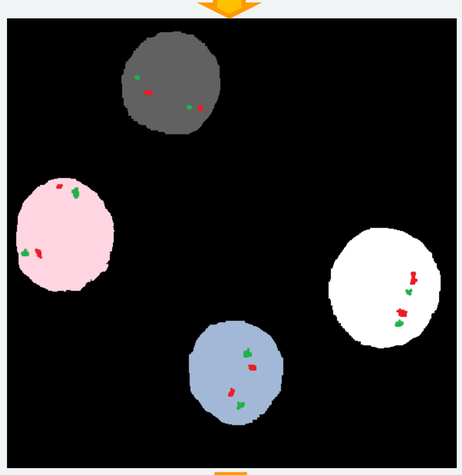
## Workflow



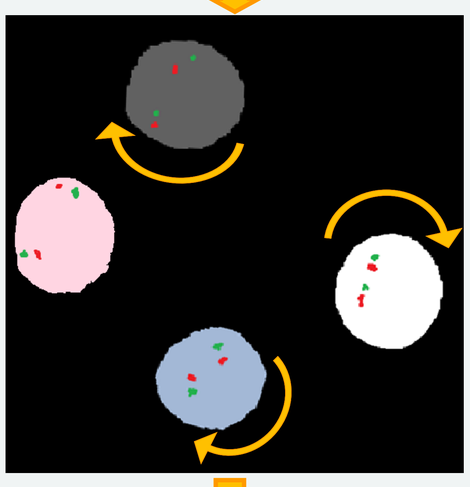
**1. Segmentation**

Identify nuclei and probes



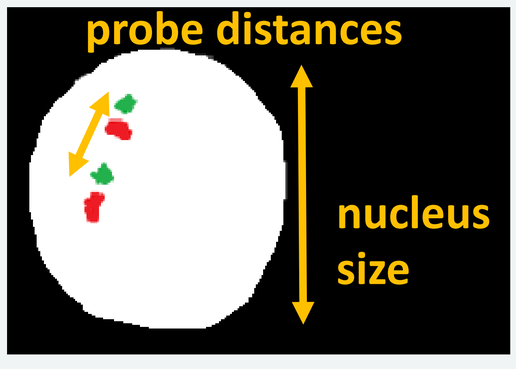
**2. Nuclei alignment**

Rotate and reverse project to 3D



**3. Nuclei statistics**

Compute properties of the nuclei population




## Introduction

Molecular microscopy and Fluorescence *In Situ* Hybridization (FISH) can be used to capture images of cell nuclei and its components. Knowledge extracted from these images about the positions of chromosomes and genes is used for medical diagnosis (cytogenetics), and can provide important insights into the spatial organization of our genome, directly or by calibrating high-throughput Chromosome Conformation Capture data [1]. In the past decade, we have witnessed significant advancements in image analysis methods. However, diagnosis still relies heavily on manual evaluation by experts, and most automatic segmentation algorithms are based on supervised approaches (hard-coded thresholds) and lack biologically driven assumptions. In addition, segmented images need to be further processed to provide meaningful quantitative measures of nuclear geometry.

## Methods and Results

We present an algorithmic workflow for transforming raw images into quantitative measures of the spatial organization of the nucleus.

### 1. Segmentation

We adopt an unsupervised segmentation method coupled with biologically driven assumptions (e.g. expected nucleus size) that can successfully segment nuclei and probes. The segmentation is a two-step process:

- **Nuclei detection**
  - ♦ we model the pixel intensities using a Gaussian Mixture Model
  - ♦ we segment the nuclei by assigning each pixel to the class (nucleus or background) that maximizes the posterior probability
- **Probe detection**
  - ♦ we compute the *à trous* wavelet components of the image
  - ♦ we detect the probes in the second wavelet component by automatically thresholding it based on the standard deviation of the coefficients

### 2. Nuclei alignment

Once the segmentation is obtained, we are interested to compute statistics about the spatial organization of the nuclei (e.g. mean size of the nucleus, radial positions of the chromosomes). However, given the heterogeneity of the nuclei and the different rotations in which they could be captured, we are first required to align and reverse project them from a 2D to a 3D space. We perform the alignment in 2D using clustering and we are currently developing a 3D alignment method based on Bayesian hierarchical models [2].

### 3. Nuclei statistics

- Average nucleus size
- Average number of probes
- Probe locations within the nucleus
- Pairwise distances between probes on the same chromosome

## Conclusions

We present a multi-method workflow for extracting spatial statistics from molecular images. We are interested to further improve the accuracy of this workflow and develop a user friendly tool for biologists.

## References

[1] Shavit Y, Hamey FK, Lio' P. (2014) FisHiCal: an R package for iterative FISH-based calibration of Hi-C data. Bioinformatics, 30, 3120-2.

[2] Green, P. J., & Mardia, K. V. (2006). Bayesian alignment using hierarchical models, with applications in protein bioinformatics. *Biometrika*, *93*(2), 235-254.