

3D SEGMENTATION AND ANALYSIS OF NUCLEAR BODIES

**Bart J. Vermolen¹, Vered Raz², Roeland Dirks², Sabine Mai³,
Ian T. Young¹, Yuval Garini⁴**

**¹Delft University of Technology, Quantitative Imaging Group,
Department of Imaging Science and Technology**

²Department Molecular Cell Biology, Leiden University Medical Centre, Leiden

**³Manitoba Institute of Cell Biology, CancerCare Manitoba,
University of Manitoba, Canada**

⁴Department of Physics, Bar-Ilan University, Israel

**Address: QI/IST Delft University of Technology
Lorentzweg 1, 2628 CJ Delft, The Netherlands
E-mail: b.j.vermolen@tudelft.nl**

The advances in nuclear molecular imaging have been immense in the last decades. Better microscopy methods (hardware) and better labeling methods (wetware) are the main reasons that we get higher quality and more detailed information. Three- (3D), four- and even five-dimensional (e.g. space (3), time (1) and spectrum (1)) imaging have become common practice. Resolution enhancements, however, give better details at the cost of significantly larger data sets. This, in turn, requires new methods in image processing and analysis (software).

We have developed a set of tools in MatLab called TeloView to process and analyze images of nuclei. TeloView is a research tool developed in collaboration with several biological research labs. After acquisition with a confocal microscope or the combination of a conventional wide-field microscope and deconvolution, TeloView is designed to process the resulting 3D FISH images of interphase cell nuclei. It can automatically segment dot-like structures (e.g. telomeres and/or centromeres) that can be interactively corrected by the operator. After this segmentation step, different kinds of analysis can be performed.

In this presentation we will describe several studies performed with TeloView. We have studied telomeres in lymphocyte mouse nuclei: we use the telomeres coordinates to calculate a parameter representing the flatness of a geometrical figure called a spheroid in which the telomeres are organized. We have shown that the telomeres organize in a disk-like structure in late G2. We have also studied human mesenchymal stem cells under activation of caspase 8 which induces apoptosis. These targets of interest were visualized with fused proteins, e.g. Trf1-DsRed and CenpA-GFP to visualize telomeres and centromeres respectively. During this process we observe a shift in centromere positions from a non-peripheral to a peripheral location contrary to the telomere shifts. To quantitatively describe this process we can segment the centromeric and the telomeric signals, localize them, and assign normalized positions, zero in the middle of the nucleus and one at the edge. We then have the radial positions of the signals and can compare radial distributions in different cell populations. This method allows us to analyze multiple targets in single and multiple cells.

Sophisticated experiments are in constant need of specifically designed image analysis algorithms. TeloView is, therefore, constantly evolving as a consequence of our collaborations with partners from biology and medical laboratories.