New FIJI Toolsets for Bioimage Analysis
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Introduction

At the imaging facility Montpellier Ressources Imagerie (MRI) we provide the creation of custom image analysis and automation solutions on demand as a service. The biologist contacts us and we find a protocol that solves the image analysis problem. If the protocol can not be executed using existing software packages, we create the necessary tools based on FIJI. The protocol and the tools are evaluated in cooperation with the biologist, using data provided by him. Once the evaluation done, the biologist uses the provided tools by himself to solve the image analysis task.

Some of the tools created in this context have been presented before, see:

http://dev.mri.cnrs.fr/projects/imagej-macros/wiki

Some of the tools like the Wound Healing Tool (Scratch Assay), the Adipocytes Tool and the Colony Blob Count Tool have been downloaded and used a large number of times with more than 23000 downloads for the Wound Healing Tool. Some of the tools recently created will be presented here.

BISE: Bio Imaging Search Engine

The Network of European BioImage Analysts (NEUBIAS - Cost Action 15124) is a unique network, aiming at strengthening the bridge between life science, computer science and digital image processing. In the context of NEUBIAS we have created an online tool, the Bio Imaging Search Engine (BISE - biii.eu). BISE allows to find image analysis workflows and seeks to provide a unification of views: problem-based (e.g. "find nuclei in cells"), method-based (e.g. "active contour based segmentation") and tool-based (e.g. "CellProfiler"). The image analysis toolsets developped at MRI are indexed in BISE.
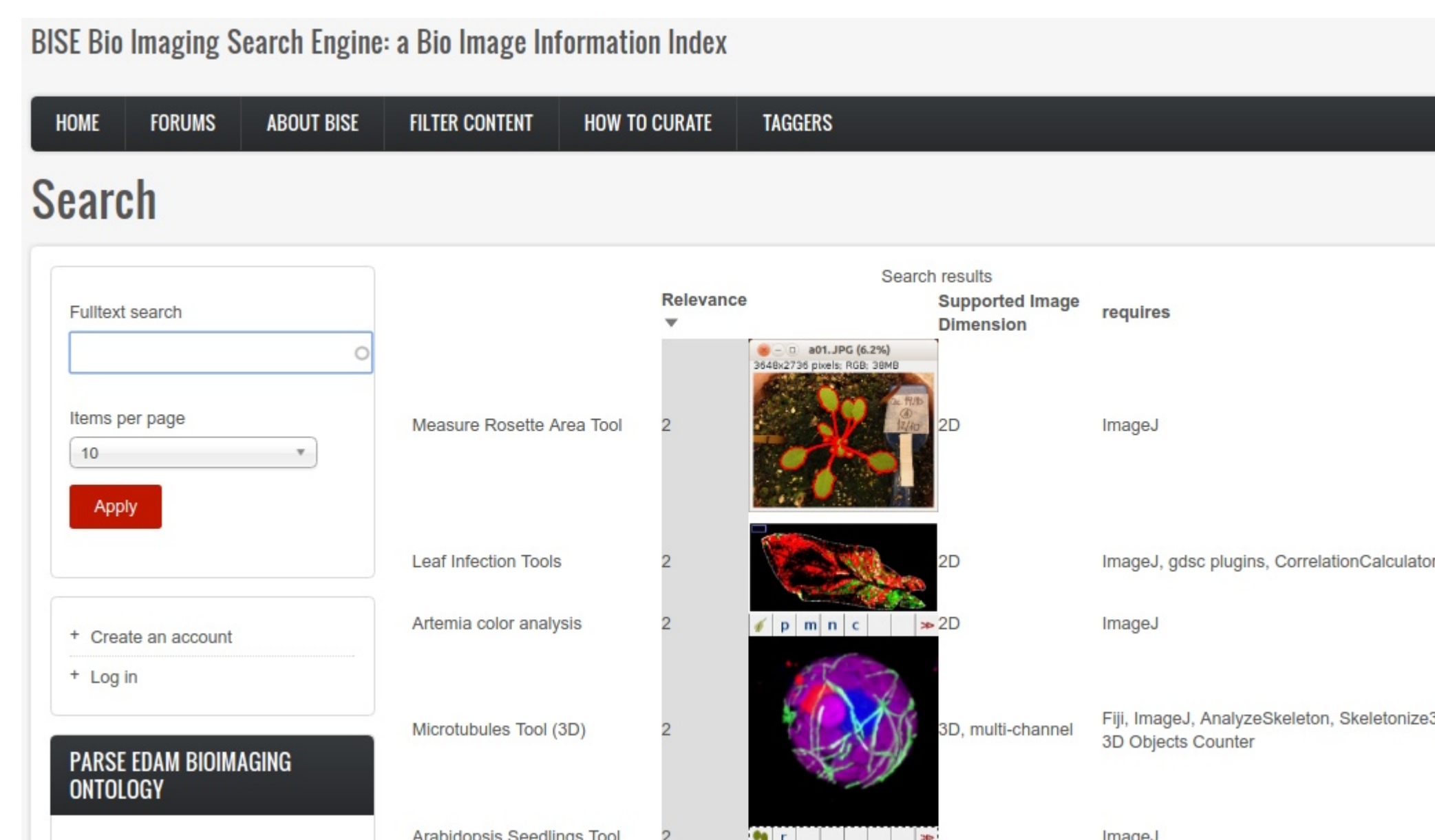
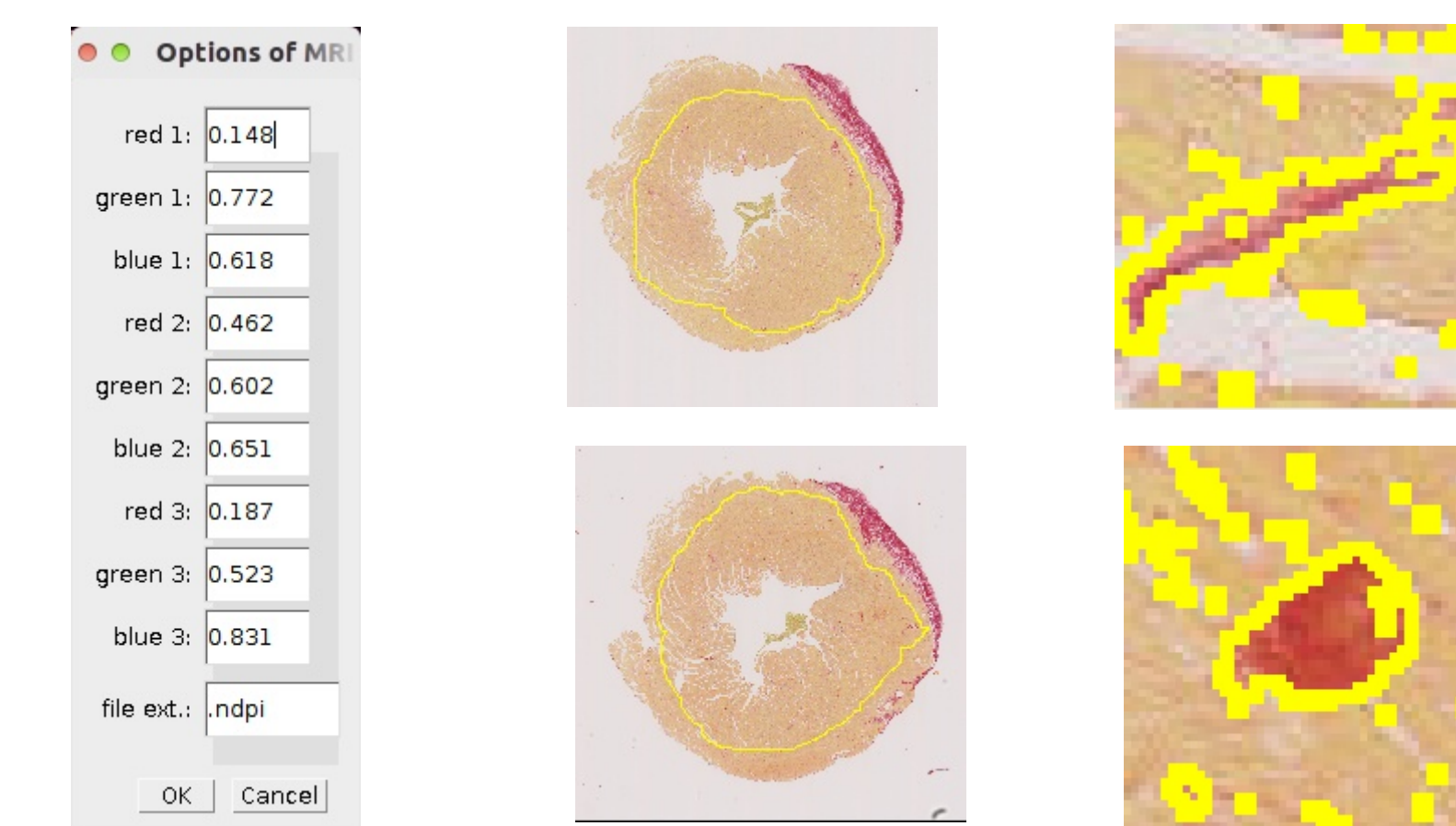## New Bioimage Analysis Toolsets

In this section some examples of macro toolsets recently developed at MRI will be described.

Analyze Spheroid Cell Invasion In 3D Matrix
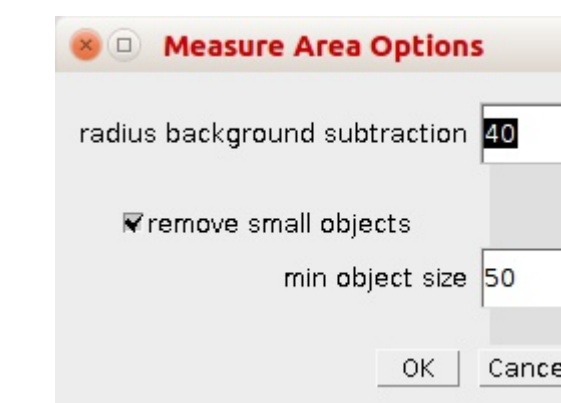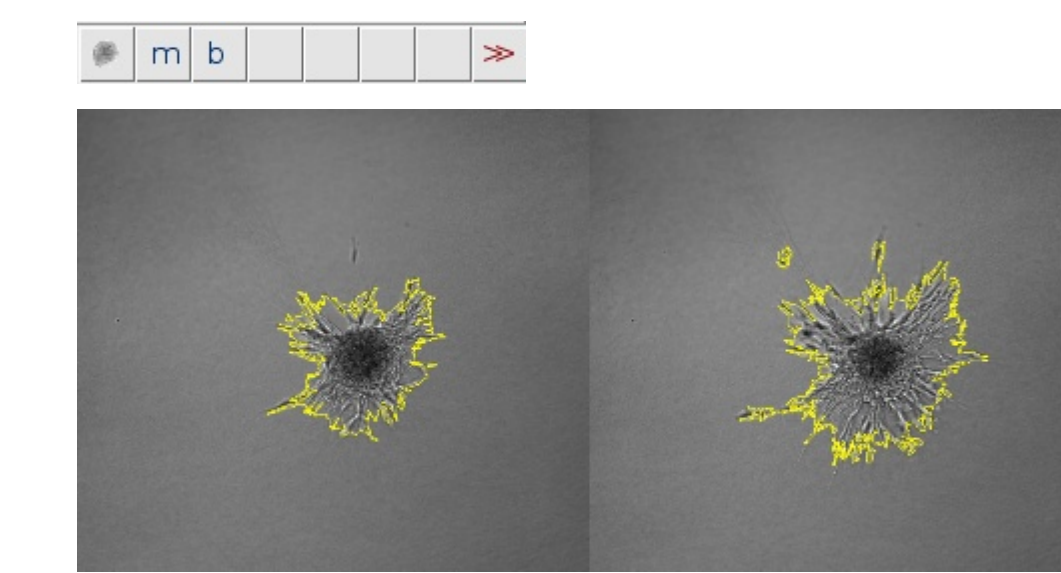
The tool allows to measure the area of the invading cancer cells in a 3D cell invasion assay. The image analysis is done on a time series of 2D-images.

MRI Fibrosis Tool

The tool measures the relative area of sirius red stained fibrosis within a selection. It uses the colour deconvolution plugin from Gabriel Landini. The tool can be used for different stainings, by adapting the colour vector for the colour deconvolution.

	title	total area	fibrosis area	relative fibrosis area
1	15010066 RS3 - 2015-04-24 18	11718831.500	100965	0.009
2	15010066 RS4 - 2015-04-24 18	12600742.800	230947	0.018

Intensity Ratio Nuclei Cytoplasm Tool
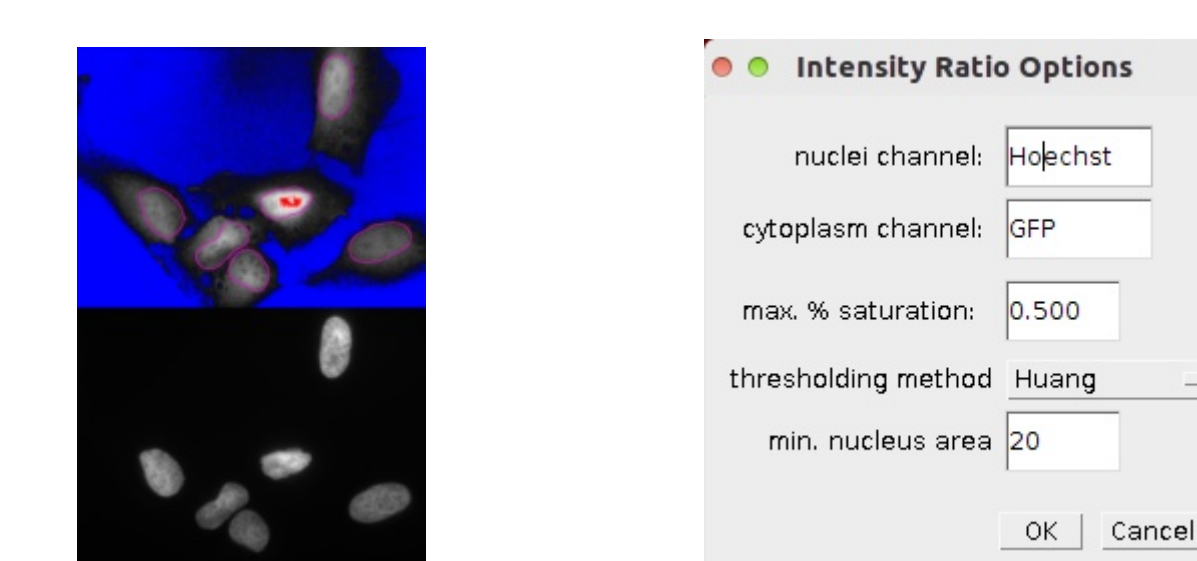
The tool calculates the ratio of the intensity in the nuclei and the cytoplasm. It needs two images as input: the cytoplasm channel and the nuclei channel. The nuclei channel is used to segment the nuclei. The measurements are made in the cytoplasm channel, after the background intensity has been corrected.

n	image	icn factor	% nuclei	% cytoplasm	av. nuclei intensity	av. cytoplasm intensity	nuclei area
1	GFP-2.tif	0.7215	41.9113	58.0887	50.2074	9.8594	96561
2	GFP-3.tif	0.9274	48.1155	51.8845	33.5035	7.8463	99138
3	GFP-4.tif	0.9395	48.4392	51.5608	37.5371	8.4065	108190

MRI Retina Tool

The aim of the tool is to measure the thickness of retinal layers. The input images are from in vivo imaging of the mouse retina with high-resolution spectral domain optical coherence tomography. By default the thickness of the the retinal nerve fiber layer together with the ganglion cell layer (RNFL/GCL) is measured.

At each given x-position of the layer, a profile plot is created. The first maximum, n-times the standard deviation above the background level is detected. A minimum to the right of this maximum, that is at least m-times the standard deviation below the maximum, is detected. A horizontal line from this point to the left is drawn and the point where it crosses the plot found. The length of this line segment is considered the thickness of the layer at the given x-position.

Jagodzinska, J., Sarzi, E., Cavalier, M., Seveno, M., Baecker, V., Hamel, C., Péquignot, M., and Delettre, C. (2017). *Optical Coherence Tomography: Imaging Mouse Retinal Ganglion Cells In Vivo*. Journal of Visualized Experiments.

Cochlea Hair Cell Counting

The cochlea is the auditory organ of the inner ear, containing inner and outer sensory hair cells, organized in respectively one and three rows inside the organ of Corti. The tool allows to count the outer hair cells in sections of 200µm from the apex of the cochlear to its base. The tool needs two types of input images: the 3d stack of the hair cells and a binary mask created from this stack by using the spot detection algorithm of Imaris (Bitplane).

The tool allows to make a projections of the two kinds of input images in batch mode. The operator then traces the cochlear on the projection of the cells image. Both images are straightened and an overlay is displayed. The operator can manually correct the image using the overlay. The cells are then automatically counted in sections of a given length.
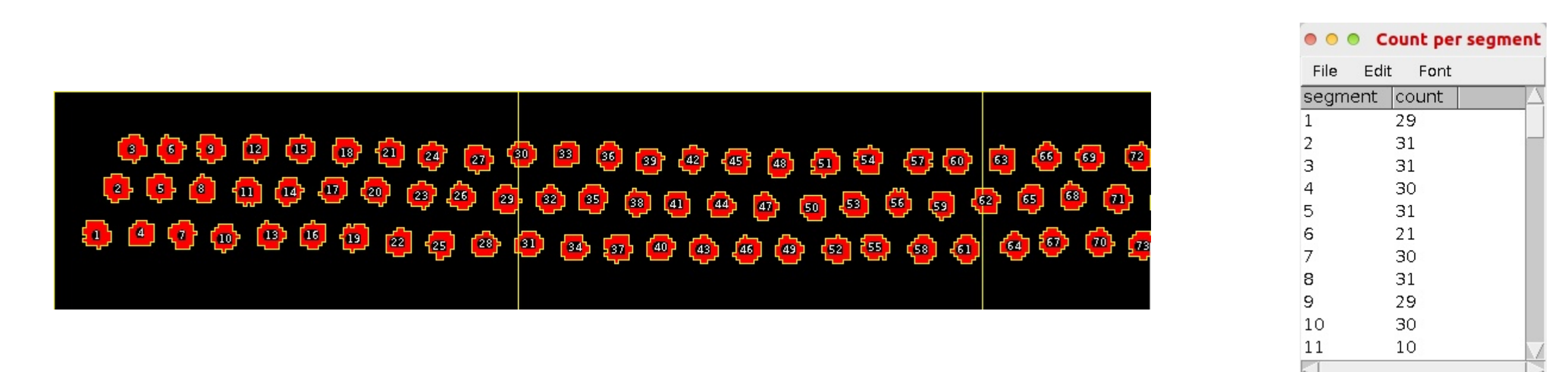
Saleur, A., Baecker, V., Dyhrfjeld-Johnsen, J., poster: AUTOMATED CELL COUNTING IN COCHLEAR HISTOLOGICAL SAMPLES, 53. Workshop Inner Ear Biology, Montpellier, 2016