Annex 6: Habilitation thesis reader's report

Masaryk University	
Faculty	MU Faculty of Informatics
Field of Habilitation	Informatics
Applicant	RNDr. Petr Matula, Ph.D.
Affiliation	Masaryk University, Faculty of Informatics
Habilitation Thesis	Automated Image Analysis in Fluorescence Microscopy
Reader	Professor Cris Luengo
Affiliation	Uppsala University, Sweden

Report Text (as large as the reader deems necessary)

Dr. Petr Matula has shown an ability to develop innovative image analysis techniques, as well as to apply these techniques in a relevant way to answer questions in biology. Over the 24 papers in this thesis, he has worked on wide breath of topics, spanning the most important imaging modalities in biology. His publications include both papers aimed at the image analysis community (J. Microscopy, Cytometry, IEEE T. Image Proc., etc.) and at the biology community (Cell Host & Microbe, Chromosome Research, Traffic, etc.). This is significant because, to me, image analysis for its own sake does not make much sense, it needs an application. Thus, scientific advances in image analysis need to enable scientific advances in another field as well. By publishing in journals aimed at both communities, Dr. Matula has shown that his work is significant both advancing image analysis and enabling other researchers to do new types of experiments.

To me, the most interesting part of Dr. Matula's work is his contribution to high-throughput screening for viral infection. Not only did he develop efficient methods for image analysis that accurately quantify the virus on a per-cell basis, but he also developed methods to judge the quality of the input images, which greatly increases the precision of fully automated screenings. This work not only resulted in four papers in highly rated journals, but also in a tool used by other projects, and which has processed about a million images so far. This type of work is impressive, and really helps advance both the fields of image analysis and biology. More often than not, image analysis tools are specifically designed for one project, and tend to be difficult to adapt to other, similar problems. The fact that Dr. Matula's tool is being used in several projects speaks highly of the effort and dedication that went into its creation.

The other work presented also is of high technical quality, with significant advances and a clear usefulness.

Dr. Matula has obviously build and extensive international network after stays in Delft, Heidelberg, and now Fontainebleau. In the papers included in this thesis, I counted co-authors from 10 different universities and institutes. Furthermore, most of his later publications do not include his former PhD supervisor as co-author. This shows a good personal development and scientific independence needed for advancement in a scientific career.

Considering all of these points, I have no doubt that this Habilitation Thesis demonstrates that

Dr. Petr Matula has sufficient technical knowledge and scientific ability to take this next step in his career.

Reader's questions to answer to defend the habilitation thesis (number of questions is upon reader's consideration)

1. In paper #9 (Cytometry A 2009) you use the intensity in the channel recording fluorescent signal from the stain for viral protein. Can you relate this intensity to an actual number of proteins?
2. In paper #12 (Review of Scientific Instruments 2011), for each object in the image, you pick its appearance from one of the rotated images, effectively creating a composite image. However, this instrument could, in principle, produce images with isotropic resolution. In papers #3 and #4 (Micron 2002, J. Microscopy 2003) you speak of fusion through either a point-wise maximum in the frequency domain, or simple averaging of the rotated images. In the computed tomography approach, where at each orientation only a 2D image is recorded, (equivalent to averaging your images along the z-axis), the volume image is computed by filtered backprojection. Comparing with your two fusion approaches, the simple averaging is equivalent to an unfiltered backprojection, and the point-wise maximum is a non-linear filtered backprojection that is sensitive to noise (or at least does not benefit from the large number of images to reduce noise). I am sure there is a better solution for this problem. What could you learn from filtered backprojection that you could apply to 3D "projections"? In particular, which regions of the Fourier space do you expect to be maximal for an image recorded at a certain orientation? Are there parts of the Fourier space that could be averaged together to reduce the influence of noise?
3. In paper #1 (J. Microscopy 2000), you fit a smooth surface through a set of points to determine the focal plane for each color. How smooth do you expect this focal plane to be? Can something in the sample preparation affect this plane?

Conclusion

Petr Matula's habilitation thesis of *Automated Image Analysis in Fluorescence Microscopy* ***does*** meet the standard requirements for a habilitation thesis in the field of Informatics.

In Uppsala on 9 July 2012

Cris Luengo