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Madrid 10 january, 2013

Dear Prof. Kratochvil,
In relation with your letter dated 31-12-2012 in which you inform me that I have been appointed as the referee for the thesis "Image Analysis in Microscopy and Videokymography" presented by Mr. Jiri Sedlar, please see attached my review.

- **Main criticism.** The thesis is composed by three subprojects. The first two consider the processing of two different imaging microscopic modalities in brightfield and AFM (atomic force microscopy) respectively. The third one is related with processing videokymographic images. The later is the more compelling and challenging project between all of them. Due to the difficulty to process such videokymograms, in my opinion, it would be better to focus the workload in such project that could have more impact in the scientific and medical community. In any case, only the first project based on the growth of filamentous specimens has produced a journal paper and the other two were presented in conferences. In relation with the third subproject, my major criticism (as I mention below in the bullet point 12) is the lack of a discriminant analysis of the large dataset of features extracted and on the other hand there is no description of the discriminatory power of the features in the case of pathological vibratory patterns e.g. through a confusion matrix.

- **Novelty.** From the methodological point of view, the 3rd subproject videokymographic present more degree of novelty than the other two that are based on more standard image processing tools (as indicated e.g. in section 2.2). Overall, in my opinion, the thesis presents enough scientific and pedagogical merits to be accepted.

- **Possible applications to other areas.** Some of the techniques described in subproject 3 are suitable to be used in other applications, e.g. in shape and texture analysis.

- **Presentation.** Some details of the described methods are spreaded in subsequent sections, but in general the presentation is fluent.

- **Other comments**

1) To facilitate the reading of the manuscript I suggest to sort the references according with the first appearance. For such purpose you can use e.g. citesort.sty package in Latex

2) Page 11: what about ground truth evaluation with control intermediate frames or even low resolution video sequences?

3) Page 30: How many images did you tested? What was the failure rate?

4) Page 32: please specify which kind of noise could be present

5) Detection of ellipses have been routinely done by the Hough transform (HT). In the thesis, there is no mention to such methods neither a comparison. It would be worth to try such comparison because HT methods could even deal with complex image data containing several overlapping and occluding ellipses. See e.g.

[1] Yuen, H.K., Illingworth, J. and Kittler, J., "Ellipse detection using Hough transform", BMVC 1988, http://www.bmva.org/bmvc/1988/avc-88-041.pdf

[2] Chia, A.Y.S. "Ellipse detection with Hough transform in one dimensional parametric space", IEEE Int. Conf. on Image Processing, pp. 333-336, 2007

[3] Chien, C.F., Cheng, Y.C. and Lin, T.T., "Robust ellipse detection based on hierarchical image pyramid and Hough transform", J. Opt. Soc. Am. A 28, pp. 581-589, 2011

5) Page 39: it would be better to use the term "centroid" instead of "core". The procedure to obtain the "cores" in page 39 is not well described. How the global threshold is determined?

6) Page 39; how the method is able to detect that "cores" lie on watershed or image borders?

7) Page 45: Is your method able to measure the heigh of surface?

8) Page 56: In any case 11 images is a small dataset for making accurate statistical measurements.

9) Page 67: How do you determine the two thresholds: t1 and t2? empirically?
On the other hand, it would be probably more efficient to replace threshold t2 by and opening/erosion morphological operation.

10) A more efficient impainting method could be based on texture modeling of the outer regions in order to avoid the "cartoon" effect that can be observed in the impainted images. See e.g.
Crimisi, A., Perez, P. and Toyama, K. "Region filling and object removal by exemplar-based image impainting", IEEE Trans. on Image Proc. 13, 2004

11) Page 80, Section 4.3.4. this section proposes a large set of parameters but there is not a clear discussion about their discriminatory power.

12) Page 89. In footnote 9 it is mentioned that videokymographic camera store the images in JPEG lossy format. Did you evaluated the impact of such compression in the final image quality?

Yours sincerely,

Gabriel Cristobal
Research Scientist