Pollen 3D: An Application of 3D Reconstruction for the Scanning Electron Microscope

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Abstract

A three-dimensional model of small size samples enables the development of accurate solutions for their conservation, education, and metrology. Due to its high magnification, the scanning electron microscope can deliver images that fit the required level of model resolution. In this work a three-dimensional reconstruction application for the scanning electron microscope, called Pollen 3D, is shown. It is based on autocalibration and its testing with images from many samples (biological, geological, and artificial) by using images recorded with two different microscopes (Auriga from Zeiss and Quattro from ThermoFisher) demonstrate its efficiency and accuracy.

1. Introduction

Overall, the sizes of pollen grains, cutting tool edge radii and ostracods vary between 7 and 150 µm, 5 and 20 µm, 0,5 to 3 mm, respectively. This level of size, and eventually the fragility, makes the education, conservation and metrology of these samples very challenging. Efficient solutions to these problems can be obtained from three-dimensional reconstruction of samples using images obtained from a scanning electron microscope (SEM), an imaging system, which resolution fits particularly well with the small size of samples.

There are many applications for reconstructing 3D point clouds from SEM images (MeX from Alicona, Austria; Mountains from Digital Surf, France; 3DSEM from Zeiss, Germany; etc.). However, it is helpful to develop a new application because the current ones, which are mainly geometry based, use the values of sample rotation taken directly from the SEM interface (Kratochvil et al., Journal of Microscopy, 2010), (Baghaie et al., Micro, 2017), (MeX, Mountains, etc.). However, experience in SEM shows that the accuracy of these values is no longer guaranteed because of errors in sample positioning with respect to the sample eucentric point. To overcome this problem, Pollen 3D computes sample rotations accurately, along with the SEM model, from an autocalibration approach (Kudryavtsev et al., Ultramicroscopy, 2020) and uses them to compute the 3D point cloud.

2. Application description

The Pollen 3D application is written in C++ with open-source libraries OpenCV, PCL and NLOpt. Before starting the application, at least three images have to be acquired by rotating the sample by about 3-10 degrees with an SEM platform or an additional robot manipulator.

The first step of the application is autocalibration. Let **Q** be a point in 3D space. It projects on the virtual SEM image plane into a point **q** according to the following affine projection:

$$\boldsymbol{q} = \boldsymbol{M}(\boldsymbol{\zeta})\boldsymbol{Q} + \boldsymbol{t} \quad (1)$$

with $\mathbf{M}(\boldsymbol{\zeta})$ a 2×3 matrix representing the upper right part of the 3×4 projection matrix $\mathbf{P}(\boldsymbol{\zeta})$ and $\mathbf{t}$ a vector representing the upper left part of $\mathbf{P}(\boldsymbol{\zeta})$, $\boldsymbol{\zeta}$ the vector stacking SEM models (intrinsic parameters) and motions (rotations and translations of the sample).

From many points of correspondence over the recorded images, we formulate parameters estimation as a problem of nonlinear optimization with constraints and use a global search approach to efficiently solve it:

$$\boldsymbol{\zeta}^{*} = \boldsymbol{min\varphi}(\boldsymbol{\zeta}) \quad (2)$$

with $\boldsymbol{\zeta}^{*}$ the value of $\boldsymbol{\zeta}$ optimizing the cost function φ defined as:

$$\boldsymbol{\varphi}(\boldsymbol{\zeta}) = \sum_{i=1}^{N_{im}} \sum_{j=1}^{N_{pt}} \boldsymbol{d}\left(\widetilde{\boldsymbol{q}}_j^i, \boldsymbol{M}_i(\boldsymbol{\zeta}) \boldsymbol{M}_i^{+}(\boldsymbol{\zeta}) \widetilde{\boldsymbol{q}}_j^i\right)^2 \quad (3)$$

where N_{im} is the number of recorded images, N_{pt} is the number of points matched, d is geometric distance, $\mathbf{M}^{+}$ is the pseudo inverse of $\mathbf{M}$. The next step deals with rectifying image pairs, i.e., making them coplanar and then easy to match. Next, a dense matching links points in pairs, and disparity maps are computed. Finally, a direct triangulation is implemented as described in (Xie, Application note, Agilent Technologies, 2011) and (Baghaie et al., Micron, 2017) to obtain a 3D point cloud that we filter out.

3. Application evaluation

Figure 1 shows three examples with their 3D models: Persicaria pollen grain (a-b), cutting tool edge (c-d) obtained with Zeiss Auriga, and an ostracode (e-f) obtained with a ThermoFisher Quattro microscope.

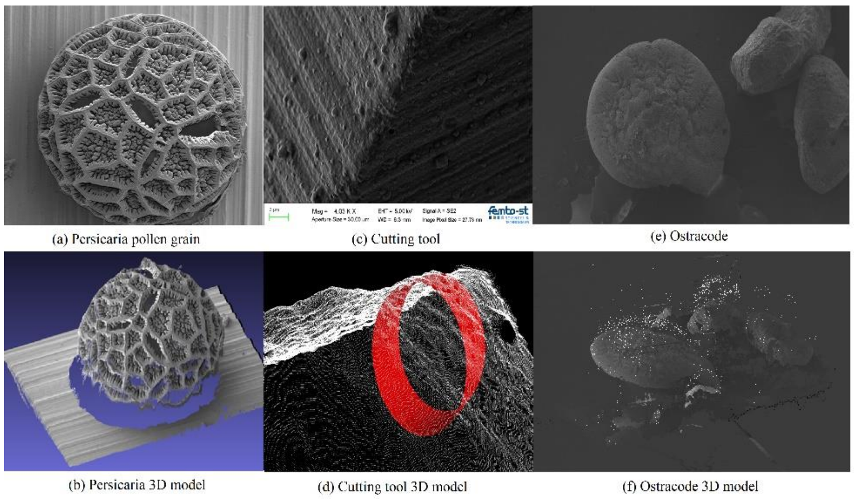

Figure 1: Persicaria pollen grain image (a) and 3D model (b), cutting tool edge image (c) and 3D model (d) and an ostracode image (e) and 3D model (f).

4. Conclusion and Discussions

Pollen 3D application is based on autocalibration of SEM, i.e., recovery of SEM model and motion directly from images of small samples to be reconstructed. Due to the quality of implemented autocalibration method, Pollen 3D gives very accurate 3D models with images from a Zeiss Auriga SEM (beam for force sensors, cutting tool edge, pollen grain) and from a ThermoFisher Quattro SEM (ostracods). Future work will focus on full 3D models reconstruction (360° rotation), indeed current are of partial, and on application of these models for education and conservation (pollen grain, ostracods, shark teeth), and metrology (cutting tool edge, powder).

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References

1. Kratochvil, B. E., Dong, L. X., Zhang, L., and Nelson, B. J. (2010). Image-based 3D reconstruction using helical nanobelts for localized rotations. Journal of Microscopy, 237(2):122–135.
2. Bradski, G. (2000). *The OpenCV Library*. Dr. Dobb's Journal of Software Tools.
3. Kudryavtsev, A. V., Guelpa, V. ,Rougeot, P., Lehmann, O., Dembélé, S.,Sturm, P., Le Fort-Piat, N. (2020). *Autocalibration method for scanning electron microscope using affine camera model*. Machine Vision and Applications 31 (2020) 1–15.
4. J. I. Goldstein, D. E. Newbury, J. R. Michael, N. W. Ritchie, J. H. J. Scott, D. C. Joy. (2017). *Scanning electron microscopy and X-ray microanalysis*, Springer.
5. J. Xie (2011). *Stereomicroscopy: 3d imaging and the third dimension measurement.* Application Note, Agilent Technologies.
6. A. Baghaie, A. P. Tafti, H. A. Owen, R. M. D'Souza, Z. Yu (2017). *3d-sem: sparse-dense correspondence for 3d reconstruction of microscopic samples*, Micron, 97, 41–55.