## Calibration of optical microscope using monodispersive polymer particles

**Background**

Fast calibration of optical microscopes for dimensional measurements including documentation is needed. Monodispersive polymer particles in a self-organized hexagonal pattern on a glass surface (objective glass) can be used as a simple means to calibrate two-dimensional length measurements on digital images from optical microscopes.

Monodispersive polymer particles (size 1 µm – 50 µm) demonstrate some attractive properties regarding the fact that they self-organize into a monolayer 2D hexagonal pattern, see figure 1. Particles are suspended in a solution of water with a low salt concentration. When a droplet containing thousands of particles is put on an objective glass and then left to dry completely, the particles lay down and attach to the glass substrate in a fixed quasi-regular pattern. Large areas of the image show a remarkably regular pattern, where the dimension of the cells of the hexagonal pattern is mainly dependent on the particle size and the homogeneity of the particles. There are always anomalies in the hexagonal pattern, with a strong dependency on the homogeneity of the size of the particles. However, the pattern gets fixed to the glass surface, and from a number of images of the same partly overlapping image area it should be possible to separate the errors in the hexagonal pattern of the calibration artefact from the intrinsic errors of the measurement system (microscope / camera).

**Objectives**

- Traceable dimensional characterisation of the reference polymer particles, and methods for statistical evaluation of size homogeneity.
- Development of MatLab code for automatic calibration of the measurement system (microscope / camera) based on sets of partly overlapping images of the same quasi-regular pattern.

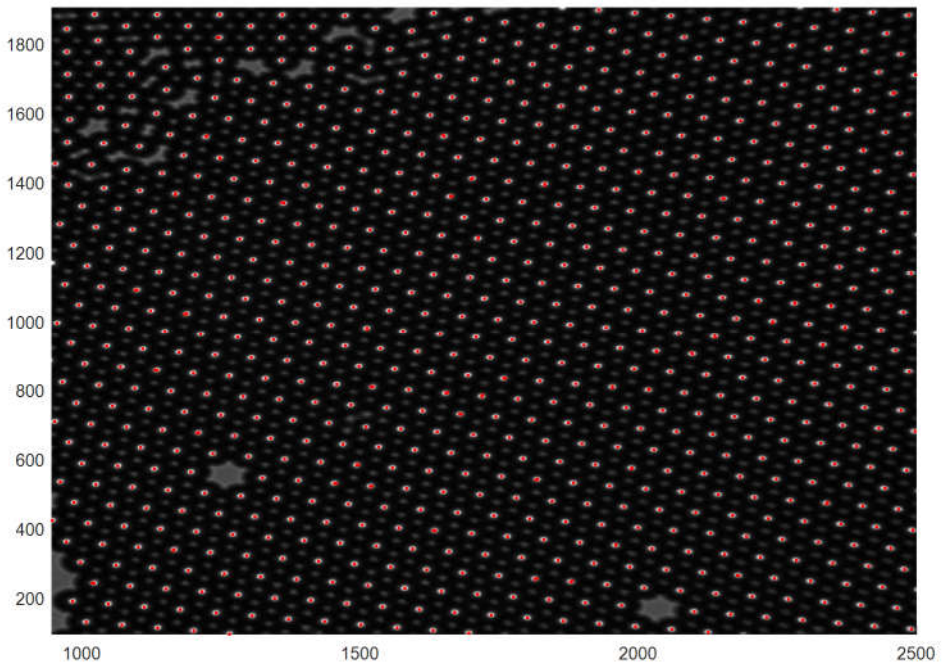


*Figure 1. Example microscope image of polymer particles, ca 10 µm diameter.*

See example image in figure 1 after identification of sphere centers in Matlab. The red dots are centers identified by a simple Matlab-algorithm:

- Threshold the image.
- Find clusters in thresholded image.
- Compute the geometric mean of the clusters and use as the location of the spheres.

**State of the art**

Today there are mainly two types of artefacts used for calibration purpose for optical microscopes:

- Stage micrometers, which are linear line scales.
- 2D artefacts, which are produced by lithographic methods, and potentially provide a large variety of fixed geometric properties.

The disadvantage of the linear stage micrometer is that it needs many points on the scale, in order to provide dense calibration information. Also, there is a need for an advanced calibration protocol in order to use the very simple geometry of a 1D linear line scale to provide traceability for all 1D and 2D geometrical properties which can be extracted from a microscope image, for example linear dimensions, straightness, angle, diameter and roundness of a circle.

A 2D fixed artefact provides directly 2D dimensions for calibration purposes, but such artefacts need individual calibration information, which is expensive.

**Industrial need**

Microbeads AS, Norway (delivers a large range of monodispersive polymer particles for calibration purpose)
Potentially all producers of imaging systems meant for dimensional measurements.

**Potential end users for the output/outcome**

Potentially all users of imaging systems meant for dimensional measurements.
Good practice guides for use of polymer particles for the purpose of calibration of microscope / camera measurement system. Calibration services for dimensional measurements will be offered at Justervesenet.

**Additional information**

The task is suitable for one or two dedicated persons for research project and master study.
The master study does not need to be done in JV's laboratory at Kjeller.

**Contact person**

Åge Andreas Falnes-Olsen
aao@justervesenet.no
988 10 363

**References**

Euramet prosjekt 1087