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PARTICLE TRACING AND ROBUST ESTIMATION OF AZIMUTHAL AVERAGES FROM CONFOCAL LASER SCANNING MICROSCOPY DATA

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ABSTRACT

Confocal laser scanning microscopy is a powerful tool for in situ observation of biomolecule chromatography. A typical experimental set up consists of a transparent micro column that is packed with porous beads and placed under the microscope. The void fraction of the column and the particle pores are initially filled with liquid buffer solution. The buffer is then pumped through the column and temporarily enriched with particular biomolecules, whose concentrations are monitored with the microscope. The molecules are transported through the column by convection and into the particle pores by diffusion.

The observation of molecule concentrations inside chromatographic particles is complicated by two issues: First, the particles are slightly moved by the applied flow, and second, the measurement data are afflicted with significant noise. We solve the second problem by exploiting the radial particle symmetry and taking azimuthal concentration averages. Our averaging method is robust with respect to reasonable variations in particle shape and position. The method is automated for the analysis of several extended image sequences without user interaction.

Figure 1 illustrates the image analysis process: First, raw measurement data is imported, and a particle of interest is chosen by the user (a). The gray levels are then converted to a binary scale with a suitable threshold, in order to determine the boundary contour of the chosen particle (b). The contours of neighbor particles are usually connected, and must be separated on the basis of particle center and size information from a previous image (c). Actual particle center and size are estimated from the resulting contour with the Fitzgibbon algorithm [1]. The resulting information is then applied for the calculation of azimuthal averages from the initial microscopy data (d).

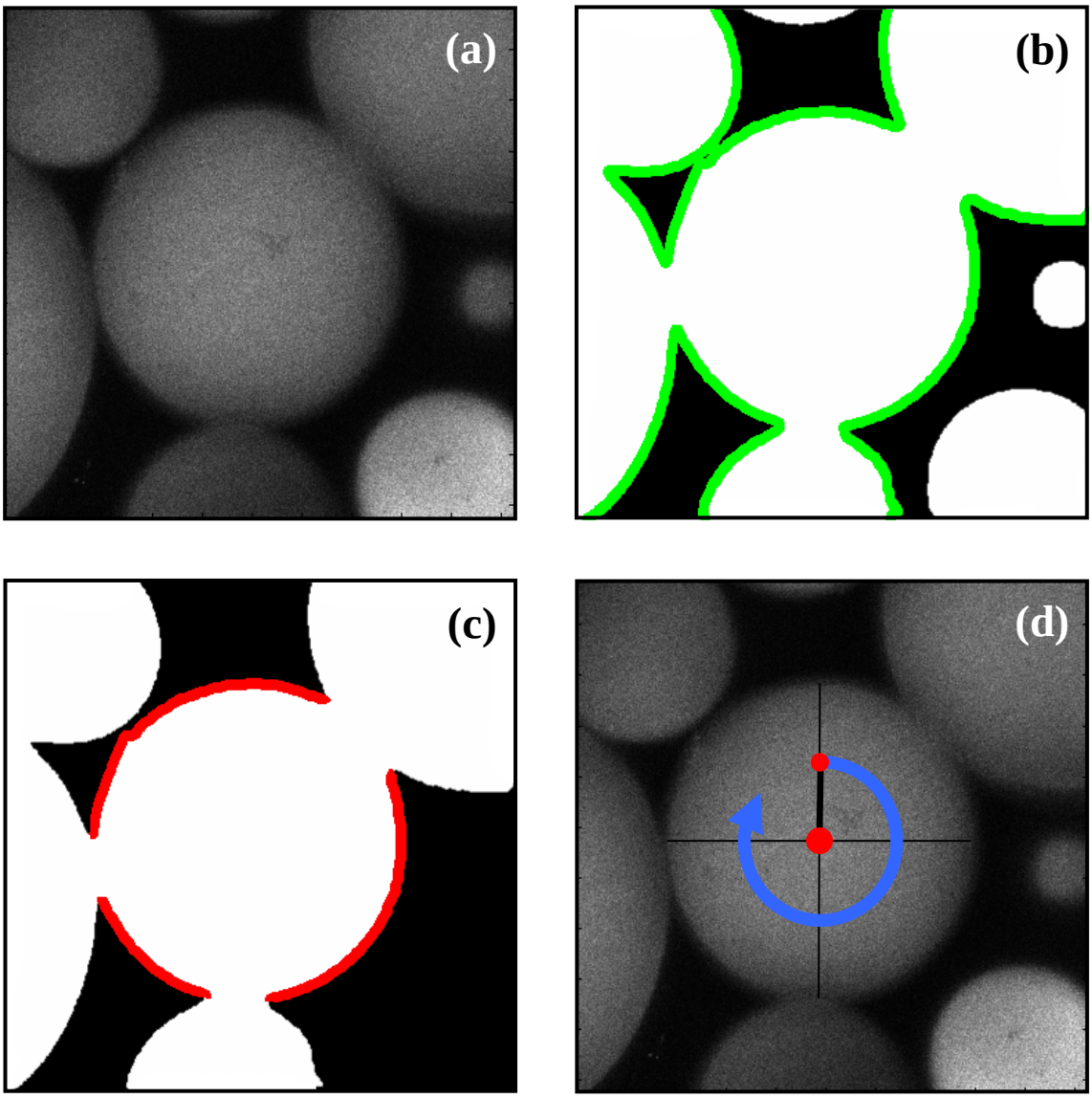

Figure 1: Determination of a particle center and of azimuthal intensity averages from a confocal laser scanning microscopic observation of column chromatography.

The concentration profiles are usually attenuated in the vicinity of particle contacts. Our azimuthal averaging procedure is, hence, designed robust with respect to outlying sectors: For each considered concentric circle, the Cartesian positions of all data points are first sorted by distance. These distances are then applied as weights for averaging the corresponding intensity values in the circle's proximity. Robustness is achieved by comparing the individual intensities with the determined mean and standard deviation. Only data points which pass this test are finally subject to a second weighted average.

REFERENCES

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