

## **Optical Method to Measure the Kinetics of Exclusion Zone Formation at Membrane-Water Interfaces and Preliminary Results**

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Does the formation of exclusion zones (EZ) at water-membrane interfaces change in response to various influences, including water activated by vortexing or human intention? There are challenges in using an open-chamber approach to studying the exclusion zone quantitatively. Thus, we designed and built a device and developed software to measure its kinetics of formation. The device is a closed flow cell chamber with 200 $\mu$ l internal volume for optimal fluid flow, comprised of two standard glass microscopic slides with a piece of Nafion<sup>®</sup> sandwiched in between them. The slides are 180 $\mu$ m apart and sealed with silicone around the edges. The Nafion<sup>®</sup> membrane was positioned in the flow cell such that its edge was centered in the field of view of the microscope. The flow cell chamber was mounted on a custom-built microscope stage composed of Plexiglas<sup>®</sup> sheets above and below that equalized the pressure on the flow cell with 4 spring-loaded screws at the corners. A polarizer and analyzer were added in some experiments. A peristaltic pump and Tygon<sup>®</sup> tubing were used to mix and circulate the water-microsphere suspension through the cell and also for washing between samples. The flow cell provided a smooth laminar flow pattern past the Nafion<sup>®</sup> membrane, as well as a vigorous flush mode to clean the chamber. When fluid motion was halted, the exclusion zone formation began, and was digitally photographed every 10 seconds for 6 min and recorded using Amscope MD Camera Series software. The micrographs were later analyzed offline with ImageJ software. The line of demarcation of microspheres was optically determined as a minimally dark point in a profile plot of a thin slice across the EZ layer of each photograph, either visually or by ImageJ software analysis. This point vs. time was plotted to yield the kinetics of EZ formation for each run. The method proved to be extremely reproducible using ultrapure laboratory Type I water with 2 $\mu$ m diameter carboxylated polystyrene microspheres. We will report on preliminary results obtained using various treatments of the water, including cosmotropic agents; vortexing; and trials with qigong masters and other persons attempting to influence the EZ formation.