

## **Laser Scanning Confocal Microscopy Applied to Multi-component Wetting**

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### **Introduction:**

Almost all previous fundamental studies of interfacial wetting, both theoretical and experimental, have focused on simple one-component fluids spreading on an inert, non-reactive surface, e.g. silicone oil spreading on a s wafer<sup>1</sup>. However, relevant systems of practical interest involve complex, multi-component blends. Consider for example adhesives, which must be spread uniformly on rough, reactive surfaces<sup>2</sup>. Additional components are frequently added to act as surface and viscosity modifiers, and flow-control agents. The extra components are believed to work by either forming a surface layer or even alloying with the surface atoms to reduce surface energy, thereby improving the spreading factor for an otherwise poorly performing fluid. On the nano- and micro- scale, the surface forces are known to dominate over bulk phenomena, effectively making many current model approaches inadequate to understand the dynamic spreading of multi-component materials.

We have proposed a multiscale approach to understanding the physics of multi-component wetting. By coupling together atomistic scale molecular dynamics simulations, continuum finite element calculations and novel experimental development, a broad range of dynamic phenomena from micro- to nano-scale are elucidated. In addition to modifying traditional tensiometry methods to work with very small quantities of fluid, we have developed a suite of experimental techniques to measure composition variations. In the remaining sections of this paper, the application of one such technique, laser scanning confocal microscopy, to study wetting of multi-component drops will be discussed.

### **Methods and Results:**

Laser scanning confocal microscopy is an advanced microscopy technique that allows much improved depth resolution relative to traditional optical microscopy. Whereas normal microscopes have depth resolutions which are many times worse than their spatial resolutions, confocal microscopes actively filter out light which does not emanate from the focal plane and thus are able to achieve drastically improved depth resolution within a factor of two of the spatial resolution for high numerical aperture objectives. The most spectacular applications of this technique have been three-dimensional images of biological structures.<sup>3</sup>

We have applied laser scanning confocal microscopy to study wetting of multi-component blends. To take advantage of the advanced imaging capabilities, each component

has been labeled with a contrasting fluorescent dye. The labeling was achieved by using a polybutadiene polymer that had been modified with reactive maleic anhydride moieties. The polymer was dissolved in tetrahydrofuran and mixed with the fluorescent label at a temperature of 50°C for one day to ensure complete reaction. The remaining fluorescent dye was removed by washing several times with deionized water and the fluorescently labeled polymer was dried in a vacuum oven for 48 hours. For these experiments, two polymers were synthesized. One polymer (A) with initial molecular weight of 3100 g/mol was labeled with AlexaFluor 555 (A30677 - Molecular Probes, Eugene, OR) and the second (B) had a starting molecular weight of 5800 g/mol and was labeled with AlexaFluor 488 (A30676 - Molecular Probes, Eugene, OR).

As a proof of concept, a coarsely blended mixture was imaged using our Leica TCS SP2 AOBS laser scanning confocal microscope. As an initial study, the fluorescence emission spectra of the two labeled polymers were measured and found to be only slightly shifted from the emission spectra available from the manufacturer. The Alexa Fluor 555 was excited at 543 nm and had its maximum emission at 555 nm whereas the Alexa Fluor 488 was excited at 488 nm with a peak emission at 514 nm. Because the two emissions were well separated, the two dyes could be imaged by specifying non-overlapping detection ranges for the two channels. However to ensure complete separation of the two signals, each dye channel was excited by their respective laser light and the fluorescence emission was scanned individually. For each image, the lines were scanned four times and averaged in order to reduce noise in the final images. Figure 1 shows images taken of the coarsely mixed blend where polymer A is shown in red and polymer B is shown in green. By overlaying the images from the two individual channels, we observe excellent separation of the two contrasting fluorescently labeled polymers and the interfaces between the two regions are quite sharp. Without the sequential scanning, there is some bleed-through of the Alexa-Fluor 488 fluorescence emission into the Alexa-Fluor 555 image.

Next we applied this technique to a well-blended polymer drop. Equal weights of the two components were dissolved in chloroform and thoroughly mixed together. The resulting blend was then dried in a vacuum oven for 48 hours. A small drop was then placed on a plasma-cleaned glass cover slip and returned to the vacuum oven for an additional 48 hours to ensure that the sessile drop had reached equilibrium. The cover slip was then placed in a custom designed holder which fits into the standard Petri dish mount on the confocal microscope. The drop was imaged through the cover slip with an oil immersion 20x objective. Since the polymer has a very similar refractive index as the glass and immersion oil, refraction through the multiple interfaces is minimized. Figure 2 shows a cross section 10  $\mu\text{m}$  from the bottom of the blended polymer droplet. In the center of the drop, the two dyes are well mixed resulting in a uniform yellow color. Near the edge of the drop, a bright green ring is apparent showing that polymer component B has preferentially coated the air interface. Figure 3 shows the relative intensities of the two dye components along a horizontal line through Figure 2. Despite some global intensity variation over the droplet, the ratio of the intensities of the two dyes stays constant through most of the drop until the air interface is reached. If the concentration is calculated assuming the bulk blend concentration in the center of the drop and that the two concentrations sum to 100%, the concentration of the green component (polymer A) is seen to increase significantly at the air-interface.

**Conclusions:**

Laser scanning confocal microscopy has been shown to be an effective tool to study concentration segregation in multi-component drops where the individual components have been labeled with contrasting fluorescent dyes. We feel this will be an important tool to help understand the physics of wetting of multi-component mixtures. In addition to discussing the progress made with laser scanning confocal microscopy, other novel experimental methods applied to study component segregation will be discussed, including Total Internal Reflection – Fluorescence Microscopy (TIR-FM) and Time of Flight - Secondary Ion Mass Spectroscopy (TOF-SIMS) which can both provide higher depth resolution down to the tens of nanometer scale to examine local behavior near the air and solid interfaces as well as near the contact line.

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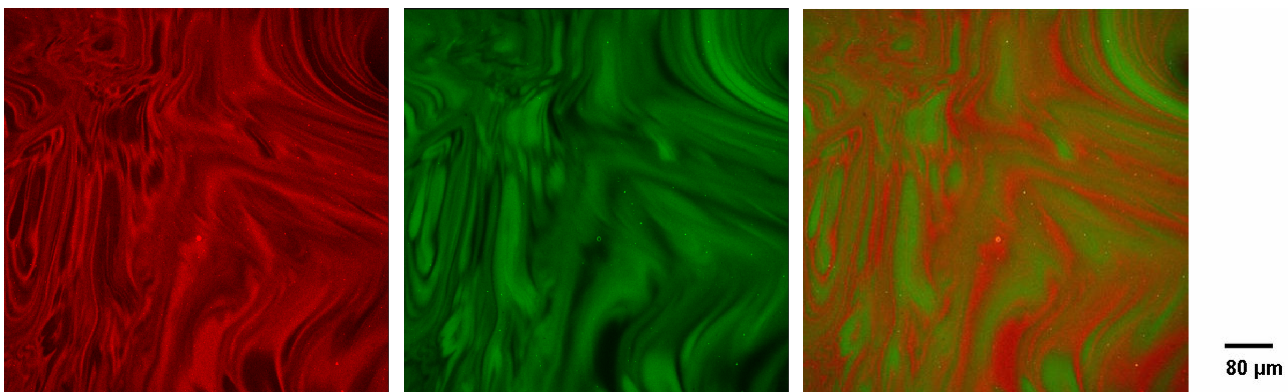


Figure 1. Confocal microscope images taken of two coarsely mixed fluorescently labeled polymers. Excellent spectral separation of the two fluorescence signals is observed.

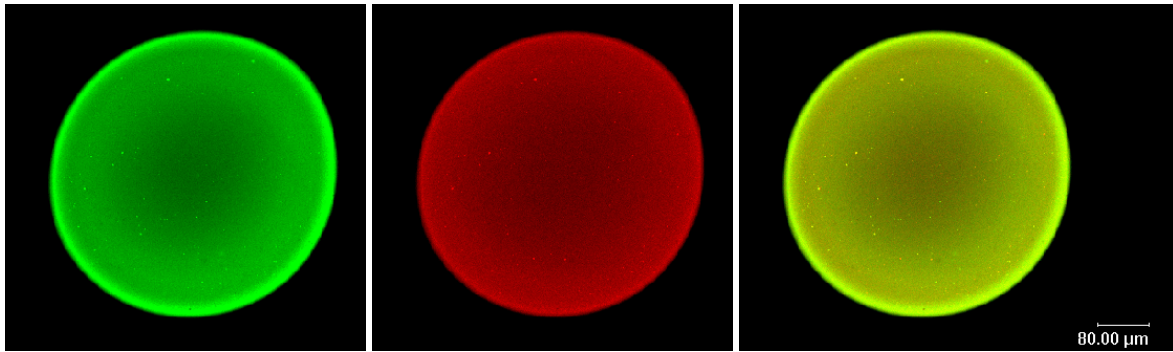


Figure 2. Confocal microscope cross-section of a sessile drop demonstrates the capability to measure concentration segregation. The well-blended region appears 'yellow' due to contributions from both 'red' and 'green' components, whereas the 'green' labeled polymer has concentrated at the air interface forming a ring.

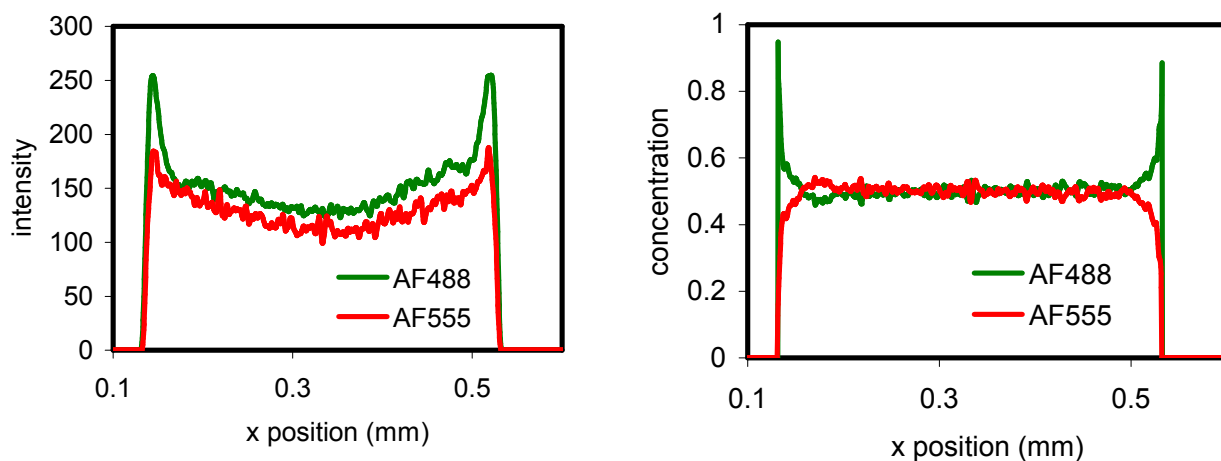


Figure 3. Cross section of drop image in Figure 2 showing the intensities of the two fluorescent components on left. Right image shows the calculated relative concentrations of the two components.

<sup>1</sup> De Gennes. Rev Mod. Phys. v57(3) (1985) p827.

<sup>2</sup> Baldan. J. Mat. Sci. v39(1) (2004) p1.

<sup>3</sup> for example <http://www.microscopyu.com/galleries/confocal/>