Techniques for Combinatorial and High-Throughput Microscopy Part 2: Automated Optical Microscopy Platform for Thin Film Research

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As it is in traditional materials science, microscopy is a central tool for morphology characterization in combinatorial and high-throughput (C&HT) materials research. Indeed, micrographs are naturally amenable to C&HT studies, since they enable rapid visual screening and automated quantitative analysis by a plethora of well-tested image processing routines. In this article, we present a custom-built and cost-effective automated optical microscopy (AOM) platform designed for the C&HT analysis of gradient specimens. Gradient specimens progressively vary one or more parameters with position, presenting a multitude of experimental conditions within a single sample (see Part 1 of this series[1]). The high-information content and planar geometry of gradient specimens make them particularly suited to AOM analysis. By combining AOM and gradient approaches, researchers at the NIST Combinatorial Methods Center (NCMC) are able to rapidly and thoroughly track the morphology and kinetics of complex materials systems over large variable spaces.[2-5] However, quantitative microscopy of gradient libraries requires an AOM system designed under specific principles. These tenets are the focus of this article. To demonstrate the NCMC AOM, and to facilitate discussion, we consider the high-temperature stability of substrate-supported polystyrene (PS) thin films. Here, we employ gradient specimens in film thickness (h) and substrate surface energy (γ) as described in Part 1.

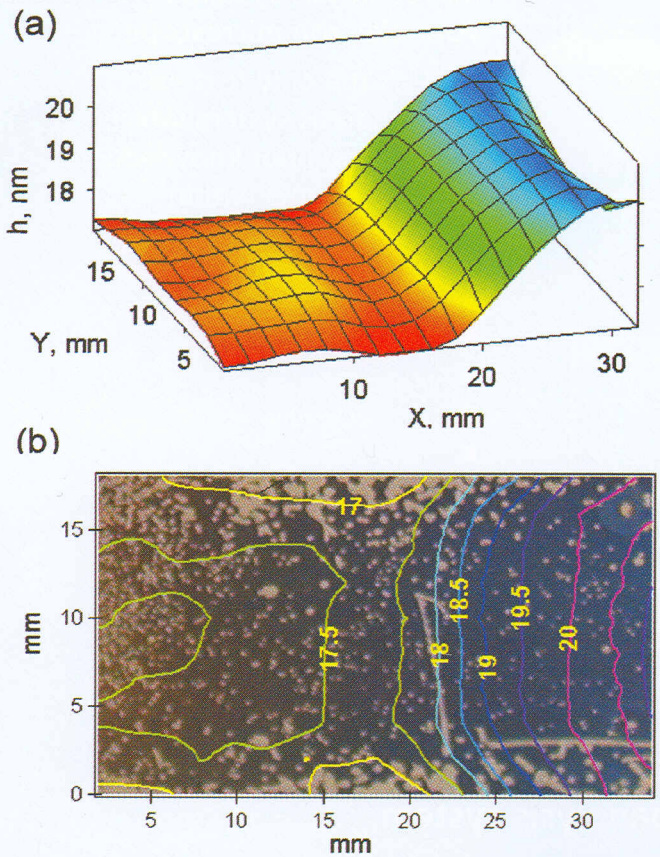


Figure 1: 2D h-gradient Specimen. A surface plot of thickness data (a) and the corresponding contour map superposed on a digital image of a h-gradient film specimen (b) show the non-linear/non-level nature of the film thickness profile.

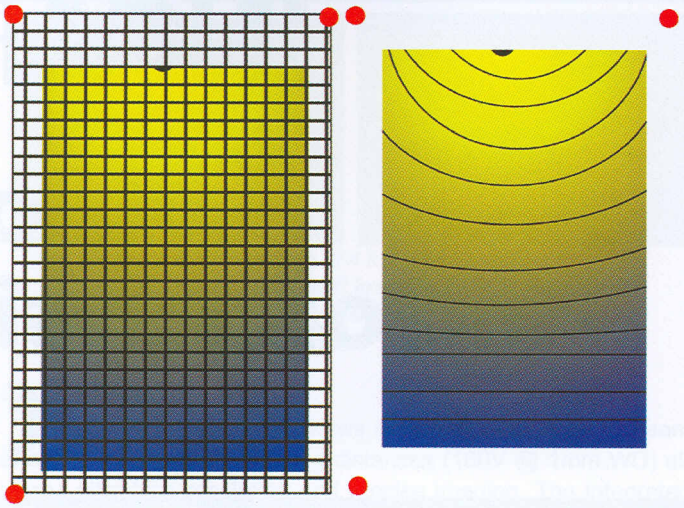


Figure 2: Generating Iso-parametric Contour Lines. 2D characterization of the gradient specimen over a tight grid of points (left) is used to generate the coordinates of iso-parametric contour lines (right). Typical routines involve coordinate sorting by parameter value followed by binning.

Design Criteria for AOM Platform. C&HT analysis of gradient specimens requires rigorous AOM design, but does not necessarily entail exorbitant expenditure on hardware. The NCMC AOM system is based on typical motorized *x-y* translation stages (Ludl‡); C&HT functionality is achieved through custom built software drivers. For gradient specimen analysis, the following capabilities were required from an AOM platform:

1) *Image acquisition over a rectilinear spatial grid.* This basic functionality involves micrograph acquisition at constant spatial increments, Δx and Δy. In the NCMC, this method is used to analyze gradients that are linear (along *x*) and that have a constant value along *y* (*i.e.* "1D" gradients, such as polymer films on surface energy libraries ($\nabla\gamma$) — *see Part 1*).
2) *Image acquisition along iso-parametric contour lines.* Non-linear/2D gradient specimens (*e.g.* the film thickness gradient, ∇h, illustrated in Fig.1) are not amenable to rectilinear image acquisition, since this strategy does not ensure correlation between parameters (*e.g. h*) and position for these samples. Non-linear gradient specimens require image acquisition along iso-parametric contour lines as shown Fig.1B. This approach facilitates parameter/position correlation and enables tracking of phenomena with respect to specific (*e.g.* iso-*h*) experimental conditions. Of course, this strategy requires prior 2D characterization of the gradient, as shown schematically in Fig. 2 and as described in Part 1.
3) *Automated focus control.* Gradient specimens can have dimensions that are ≥ 5 cm, and level mounting of specimens on the microscope stage is rarely guaranteed. Accordingly, an AOM generally requires automated focus control, especially when using high magnification (*i.e.* 20X and above). Automatic focusing is also necessary for kinetics studies, where the microscope may be acquiring images over many hours and where manual focusing at each step is impractical.
4) *Timed acquisition for kinetics measurements.* C&HT microscopy studies of morphology evolution require an AOM system that monitors both the time and position of acquired images. As seen below, this requires coordination between the position, focus and image acquisition in the AOM system.

AOM platform design. LabView®, a graphical programming language for instrument control and data aquisition, was used to construct the NCMC AOM system. A graphical interface (front panel, see Figs.3,[4]) allows for input of operational parameters. The graphical interface is linked to a series of instrument-directing subroutines, or block diagrams,

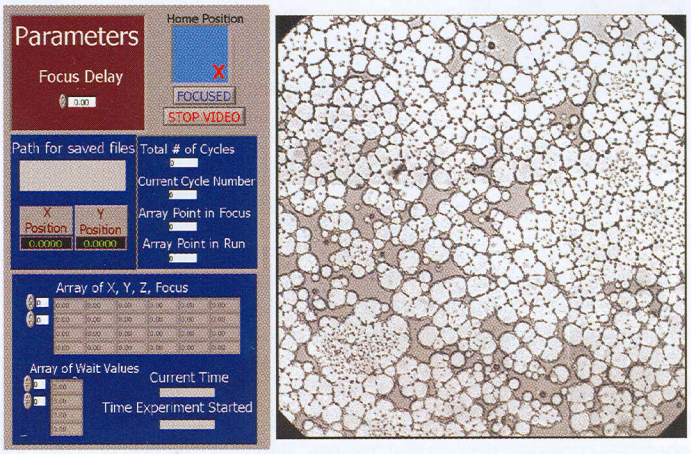
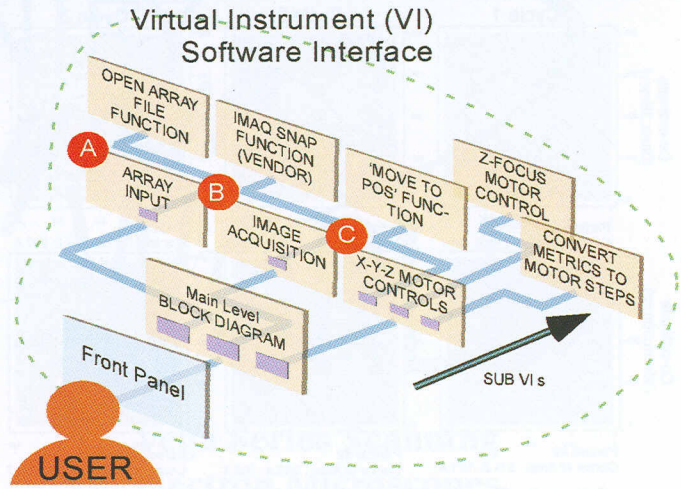


Figure 3: The graphical interface or front panel for the NCMC AOM platform. Image (right) shows the morphology of a dewet PS film.

that are organized into a so-called virtual instrument (VI, see Fig. 4). The VI controls the microscope *x-y* stage and *z*-axis focus motors, which are connected in parallel through commercial control hardware to the serial I/O port of the AOM computer. The VI also coordinates image acquisition by a CCD camera (*e.g.* a Hitachi VK-C350). In the following paragraphs, the VI structure is discussed in context of the design criteria.

1) *Incremental array scan.* The NCMC AOM system can collect images at constant increments of Δx, Δy, and time (Δt). In the main level of the VI, *x-y* stage motors are commanded through a vendor-provided (Ludl) Labview function "Motor Move to Pos" (Fig. 4C). An IMAQ subroutine, "Imaq Snap", triggers image acquisition on completion of motor translations (Fig. 4B). The VI prompts incremental step distances, number of steps in *x* and *y*, number of imaging sequence cycles, Δt between cycles, and a focus delay (δt) that allows the user to focus the camera at each imaging step (optional).
2) *Scan along iso-parametric contour lines.* Image acquisition along iso-parameter contour lines (Fig. 1B) requires input of *x-y* coordinates derived from 2D characterization (*e.g.* spot interferometry) of the gradient specimen (see Fig. 2). In the NCMC AOM system, the Labview "Read From Spreadsheet Array" routine reads contour-line coordinates from a user-specified file (Fig. 4A). These *x-y* positions are used as the input to the "Motor Move to Pos" routine (Fig. 4A) with image acquisition at each point using "Imaq Snap".
3) *Z-focus control.* The NCMC AOM uses a *z*-focus module for focus control. This device uses an encoded motorized clutch to drive the course focus knob; this clutch is connected to the AOM computer through the serial port. The *z*-focus module is controlled through the "Serial Port Write" subroutine, which utilizes the "where *f*" and "move *f*=" commands to read the current encoder position and move the motor to a specified position (respectively, see Fig. 4C). Instead of an "autofocus" routine (which can be unreliable and time consuming), the NCMC AOM achieves focus control through a "learning step," which is executed with the specimen in place but before the actual experiment. The "learning step" routine visits the positions at which micrographs will be captured. At each (*x,y*) point, the user focuses the image and the AOM system reads the *z*-focus module encoder position and writes it to an array. This array of *z*-positions is then used to drive the *z*-focus module throughout the experiment.
4) Kinetic measurements. The NCMC AOM enables the study of morphological evolution over hours or days without user intervention. Image acquisition timing is controlled both between micrographs and between execution cycles using the "tick count" function (Fig. 3, Fig. 4B). Moreover, the user can input a series



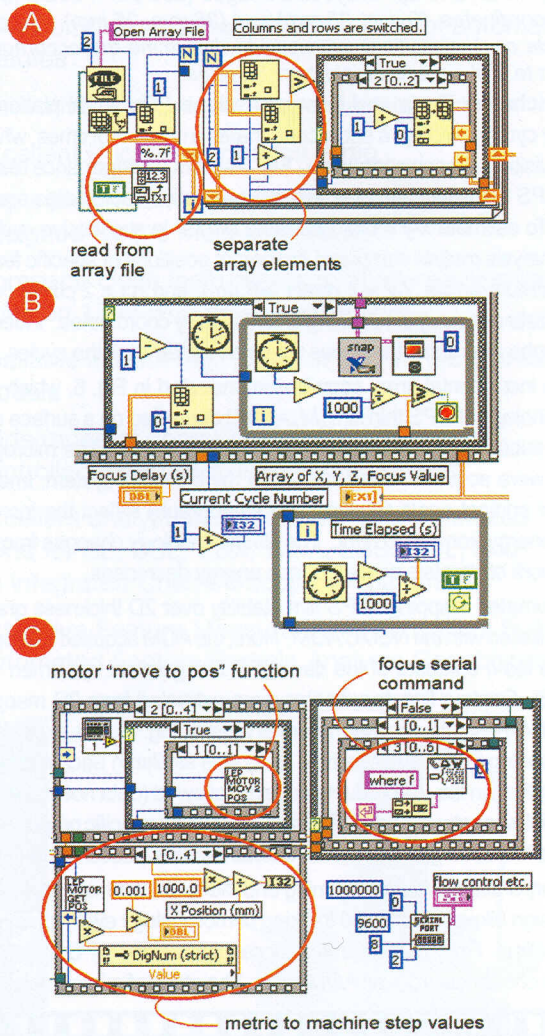


Figure 4: Schematic representation of the AOM platform software. Subroutines that direct microscope functions are organized into a virtual machine, or VI. (A) Subroutine for input and parsing of coordinate arrays for image acquisition along iso-parametric contours. (B) Image acquisition and timing routine (C) Motor control routine for coordinating x-y translation stage motion and z-focus module operation.

of "wait" intervals such that, for example, successive acquisition cycles occur after increasingly longer waits (Fig. 3). Such a strategy allows for decelerating kinetic processes to be tracked in a rational manner.

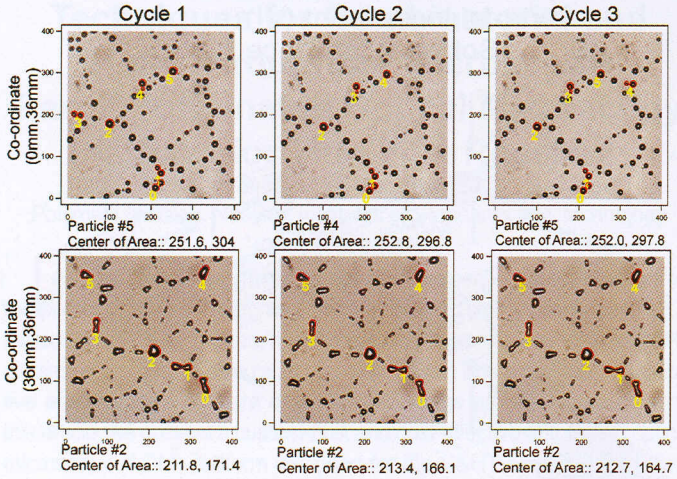


Figure 5: Benchmark test for quantifying x-y stage "following errors" in Δx and Δy. Grayscale images (292 μm x 292 μm) for two coordinates (0 mm, 36 mm) and (36 mm, 36 mm). Labeled particle center positions are used to determine the accuracy of return to a given coordinate over 3 cycles.

Benchmark Tests and Demonstrations. The AOM platform was tested by cycling through a set of coordinates a number of times, which image acquisition at each coordinate. Fig. 5 shows a performance test using a dewet PS film specimen over two representative coordinates and three cycles. To estimate *x-y* motor "following errors" in successive cycles, an image analysis routine compared the center positions of specific features. The maximum errors, $\Delta y \approx 7$ pixels (≈5 μm), and $\Delta x \approx 2$ pixels (≈1 μm) demonstrate accuracy in returning to specific x-y coordinates. Indeed, the micrographs also show that focus was maintained over the cycles.

The incremental array scan is demonstrated in Fig. 6, which shows the morphology of a PS thin film (M_r = 28K) deposited on a surface energy gradient substrate and annealed for 60 min at 140°C. The micrographs in Fig. 6 were acquired along the 1D $\nabla\gamma$ by the AOM system. Images of the water contact angle (above each micrograph) reflect the increasing surface energy from left to right. The film morphology changes from holes to a network of droplets as the surface energy decreases.

Automated mapping of PS film stability over 2D thickness gradients was also tested with the NCMC AOM. Here, the AOM acquired micrographs along the iso-*h* contours of the dewet PS film gradient specimen shown in Fig. 1B. Contour line coordinates were extracted from 2D mapping of the film thickness via spot-interferometry (as in Fig. 2). As expected, the film morphology (*e.g.* average hole size) was similar in each micrograph, indicating that an iso-*h* contour was being followed (data not shown). The ability to follow contour lines is important, as it allows specific properties to be tracked in "crossed gradient combinatorial libraries", described in Part 1.

Incorporation of the cycle timing and the focus "learning step" into the AOM design allows automated tracking of morphology evolution over long periods of time. Fig. 7 demonstrates successful tracking of PS film rupture at a specific coordinate from an AOM scan. The string of micrographs shows

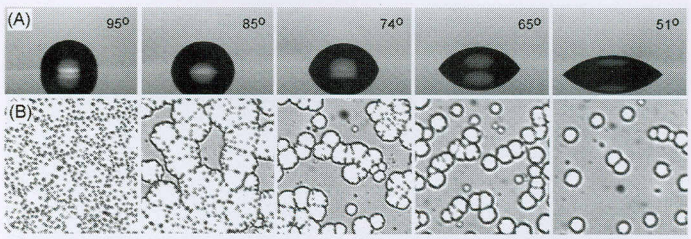


Figure 6: PS film on a 1D Surface Energy Gradient. Micrographs of PS film on substrate with increasing surface energy (left to right) as reflected by water contact angle measurement (above micrographs). The images were acquired at equal intervals over 36 mm on a single specimen

the formation and impingement of holes, and the subsequent breakup of the film into droplets. The power of AOM for kinetics studies is revealed when one considers the fact that "movies" like Fig. 7 can be generated for each point in the gradient library. Accordingly, kinetic data is being collected over a wide range of experimental conditions.

Conclusion

The development of Automated Optical Microscopy systems is essential for C&HT investigations in materials science. In this article, we demonstrated a simple, cost effective, custom-built AOM system designed for the analysis of gradient specimens. The system is built using a basic set of functions that coordinate stage translation, image acquisition, cycle timing and focus control. Especially, when complimented by automated image analysis[6], AOM represent a powerful tool for the rapidly and efficiently mapping of materials behavior and for accelerated materials discovery.

For more information on this AOM system and C&HT methods visit the NIST Combinatorial Methods Center website at http://www.nist.gov/combi, or contact the NCMC by email: combi@nist.gov. ■

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Footnotes

† An intern from Montgomery Blair High School, Silver Spring, MD 20901

‡ Certain equipment and software are identified in order to specify experimental details. Such identification does not imply recommendation by the National Institute of Standards and Technology, nor does it imply the equipment is necessarily the best available for the purpose.

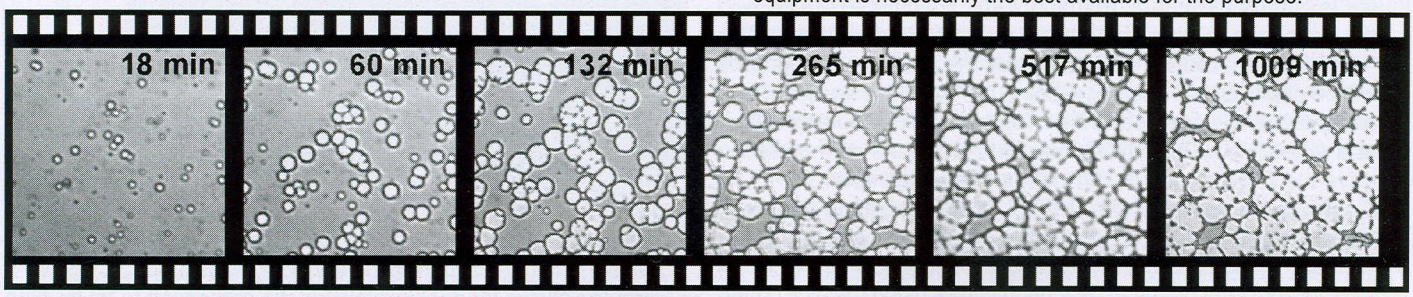

Figure 7: Time evolution of PS film structure at one co-ordinate. Similar data automatically collected over an array of (x,y) points provides kinetics data over many conditions (for example γ and h) using a single gradient library specimen.