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Nano-patterned standards for improving the quantitative capability of laser scanning confocal microscopy for materials characterization

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Abstract

We explore novel means for estimating the sizes of features that lie below the diffraction limits for laser scanning confocal microscopy (LSCM) for non-destructive, quantitative materials characterization. We first employ electron beam lithography to fabricate calibration standards consisting of hollow, nanometer-scale features as small as 70 nm in diameter etched in a poly(methyl methacrylate) photoresist. In addition to a range of feature sizes (D), we have designed these standards to also explore resolution limits through various feature-to-feature spacings. Fluorescence mode LSCM imaging of the features (i.e., saturated with fluorescent dye), and measurements via scanning electron microscopy (SEM) enable us to correlate the fluorescence intensity with the size of the sub-resolution features. Finally, we leverage this calibration for more quantitative interpretation of LSCM images of polycrystalline NaX zeolite membranes.

Keywords: Laser scanning confocal microscopy (LSCM); Calibration; Polycrystallinity; Zeolite membranes; Electron beam lithography

1. Introduction

A common challenge in materials characterization is the ability to *quantitatively* and *non-destructively* characterize the size and distribution of structures, defects, and interfaces (e.g., between phases or materials) hidden beneath material surfaces. In fact, a number of recent technology roadmaps [1–3] have targeted quantitative analysis of such buried material interfaces as a critical area for current nanomaterial initiatives. This focus is, in part, motivated by a need to establish structure–properties relations for these systems, especially when integrated in devices where such interfaces can affect device performance.

In Refs. [4,5] we have discussed the application of laser scanning confocal microscopy (LSCM) as a novel means for characterizing the buried polycrystallinity of zeolite thin films. For those systems, and ultimately for many materials of interest from the nanotechnology perspective, we are interested in characterizing the spatial distribution and size of features at the nanometer length scale. To this end, a shortcoming of LSCM arises from its optical nature and its diffraction limited resolution. Indeed, this technique could be more effectively leveraged for quantitative nanomaterials characterization by developing techniques to extend its resolution limits to smaller scales.

The fluorescence from a single point source measured by optical techniques is in the form of a radially decaying distribution, termed a point spread function (PSF) or Airy disc. In addition to a strong central peak, optical diffraction gives rise to decaying subsidiary peaks. The well-known Rayleigh criterion [6] suggests that in widefield microscopy two adjacent, infinitely small point sources can be resolved ideally only when the distance between them is at least an Airy radius, r_{Airy}, given by

$$r_{\text{Airy}} = 0.61\frac{\lambda}{\text{NA}}, \tag{1}$$

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where NA is the numerical aperture of the objective and λ is the wavelength of the emitted photons. This radius corresponds to the distance between the center of the diffraction pattern and the first diffraction peak. The lateral resolution is improved in confocal microscopy to

$$r_{\text{lateral}} = 0.4\frac{\lambda}{\text{NA}} \tag{2}$$

as a result of a reduction in the intensity of the subsidiary diffraction peaks beyond the central peak [7] of the diffraction pattern. This lends insight into the ideal resolution for confocal microscopy, suggesting that, for example, a larger NA of 1.2 and a 560 nm wavelength light would give rise to a lateral resolution of approximately 0.2 μm.

This sub-micron lateral resolution underscores how LSCM can be a useful technique for characterizing porous materials with micron-sized pores, and begins to underscore the challenge for quantitative characterization of materials with smaller, denser features. It does not, however, say that fluorescence from small features is unobservable; rather just that one sub-resolution feature cannot be quantitatively differentiated from another based upon standard image analysis. As such, in this work we focus our attention on means for extending the resolution limits of LSCM by novel image calibration.

Conventional image calibration efforts within the literature have considered small, sub-resolution objects in order to experimentally evaluate the resolution limits and PSF of particular instruments for comparison to theoretical predictions. These types of studies can be respectively classified in terms of the calibration materials as either grating-type or fluorescent microsphere studies.

The former studies typically focus on understanding resolution limits. As discussed in [8], these types of approaches involve etching periodic patterns of parallel lines with a range of sub-micron periodicity in a thin (e.g., aluminum) film on one side of a coverslip. When mounted in a dye solution for imaging, this orientation facilitates diffusive exchange of dye in the features with the bulk mounting solution to minimize issues associated with photobleaching. Since the aluminum layer is opaque, only fluorescence from dye in the etched regions is observed via LSCM.

The latter micro or nanosphere studies (e.g., [9–14]) are commonly employed within the literature to experimentally measure PSFs. Latex microspheres of calibrated size and stained with different fluorescent dyes are typically used. Such microspheres are commonly developed for instrument calibration and are designed to be relatively resistant to photobleaching. One of the most significant differences between these methods and the grating studies described above is the nature of the fluorescing medium measured by LSCM. In particular, while essentially a pool of fluorescent molecules is imaged in the grating studies, in the bead studies, fluorescent molecules are randomly distributed throughout the latex matrix.

Ultimately, we are interested in calibrating images of material polycrystallinity in which dye molecules are occluded in void features throughout a transparent sample (e.g., see Fig. 1). In particular, we aim to quantitatively measure the size of these features. The *dispersion* of the dye within the polymeric matrix in bead studies makes direct correlation of fluorescence intensity with the *occluded* dye in polycrystalline features difficult. A more direct comparison could be drawn between our materials characterization studies and the grating studies described above because of the comparability of the dye conditions (i.e., dye-filled features rather than dispersion in a polymeric matrix). The grating studies, however, employ an *opaque* aluminum mask in comparison to the three-dimensional *transparent* single-crystal grains separating polycrystalline features (voids) in our studies.

Motivated by the limitations in direct applicability of common calibrations to our system, we have developed a new calibration standard with hollow, point like features patterned in a transparent film. The novelty of the new standard results from its partial recreation of the environment of interest (i.e., sub-resolution reservoirs filled with dye molecules). In addition, we use these new standards to evaluate features with sizes below those probed by common grating studies.

We describe these image calibration techniques in this paper. First, we discuss the electron beam lithographic construction of nano-patterned standards consisting of hollow features of a range of diameters and feature densities etched in a transparent material (i.e., polymeric photoresist). Subsequently, we describe LSCM and SEM analysis of these standards. We discuss means for analyzing the fluorescence signal collected from these standards for comparison with SEM measurements of feature size. This analysis yields a calibration-based means for measuring sub-resolution features via the fluorescence intensity emitted from fluorophores occluded within these features. Finally, we turn our attention to the practical example of NaX zeolite membranes, and develop a calibration curve for linking feature size and fluorescence intensity in that system.

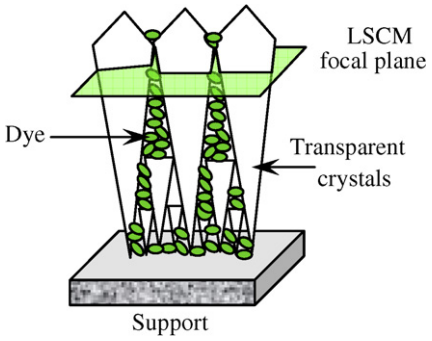

Fig. 1. Schematic of the intergrown crystal structure of a c-oriented silicalite-1 zeolite membrane grown on a porous α-alumina support, with polycrystalline features saturated with fluorescent probe molecules.