A new order of nanoplastic standards for microspectroscopy calibrations

Andrew C. Madison, Daron A. Westly, B. Robert Ilic, Craig R. Copeland, Adam L. Pintar, Charles H. Camp, J. Alexander Liddle and Samuel M. Stavis*

*†National Institute of Standards and Technology, Gaithersburg, Maryland 20899, *samuel.stavis@nist.gov*

Plastic nanoparticles are of increasing interest and concern. Nanoplastic products enable commercial applications ranging from optical probes[1] to drug delivery,[2] whereas the unintentional release of nanoplastic byproducts into the environment poses potential hazards of chemical sorption and tissue penetration.[3] Optical microspectroscopy is essential to characterize nanoplastics[4] but lacks standards that are fit for purpose. Rayleigh scattering, fluorescence emission, and Raman scattering signals all vary with nanoparticle properties,[5, 6, 7] while imaging systems present multiple aberrations,[8] limiting accuracy and requiring reference values of nanoplastic properties, as well as reference positions. The latter issue is unexplored in this context, motivating nanoplastic standards with new spatial order.[5] To address all of these issues, we introduce the concept of the nanoplastic array (Figure 1). This prototype standard enables calibration and correlation of image data from multiple instruments, improving dimensional and optical metrology of nanoplastic samples. We expect that nanoplastic arrays will enable new accuracy and unity of optical microspectroscopy, advancing the quantitative measurement of nanoplastics to optimize product performance and to understand byproduct hazards.

To prove the concept, we fabricate nanoplastic arrays in thin films of phenolic resin, containing fluorescent dopants, by electron-beam lithography. Our nanoplastic arrays (Figure 1a-c) feature three types of nanostructures, building in complexity and functionality, and enabling multiple calibrations. First, a uniform film enables correction of non-uniform irradiance for the accurate analysis of fluorescence and Rayleigh scattering intensity, and provides reference spectra for Raman measurements (Figure 1d). Next, a uniform pillar array with diameters of less than 1000 nm provides a repeating reference dimension and regular reference positions for microscopy modes of electron scattering for size validation (Figure 1e), and Rayleigh scattering (Figure 1f), fluorescence emission (Figure 1g), and Raman scattering (Figure 1h) for optical calibration. Last, variable pillar arrays with diameters ranging from 150 nm to 1000 nm in a repeating raster pattern facilitate systematic measurements of nanoplastic properties as a function of size. The smallest features of our nanoplastic arrays resemble point sources for calibration of instrument response through focus (Figure 1i-j). Fine gradations of the diameter of nanoplastic pillars enable quantification of the limiting signal-to-noise ratio at which detection of nanoplastics is possible (Figure 1k-l). Our new order of nanoplastic standards begins to meet the urgent needs for suitable reference materials.

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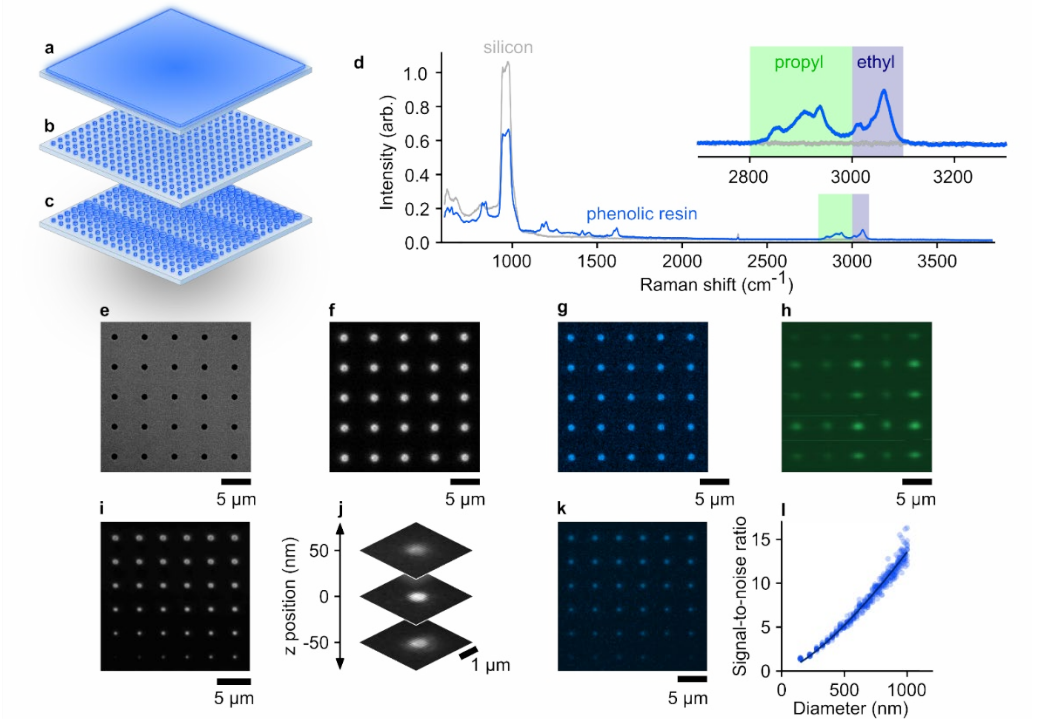

Figure 1. Structures and functions of a nanoplastic array. (**a-c**) Schematic of three types of nanostructures, including (a) a uniform film, (b) a uniform pillar array with diameters of less than 1000 nm, (c) a variable pillar array with diameters from 150 nm to 1000 nm in a repeating raster pattern. (**d**) Representative Raman scattering spectrum of a uniform film of phenolic resin on a silicon substrate. (Inset) Spectral bands of (green) propyl and (blue) ethyl groups forming the phenolic resin. (**e-h**) Micrographs of various optical responses of the uniform pillar array with diameters of less than 1000 nm, including (e) electron scattering, (f) Raleigh scattering, (g) fluorescence emission, and (h) Raman scattering. The Raman image in (h) maps the spectral bands of propyl groups, 2800 cm^{-1} to 3000 cm^{-1}, to the green channel and ethyl groups, 3000 cm^{-1} to 31000 cm^{-1}, to the blue channel of the image. (**i-l**) Optical responses of the variable pillar array, including (i) Raleigh scattering, (j) instrument response to a sub-resolution pillar around best focus, (k) fluorescence emission, and (l) representative fluorescence signal-to-noise ratio as a function of pillar diameter, testing the limit of detection of our optical microscope.