Imaging Color Target for Off-Axis Illumination Reflectance Microscopy

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Abstract

A color target has been designed and fabricated for off-axis illumination reflectance microscopy that utilizes microlens arrays and color mirrors. The color mirrors are optical interference coatings that are similar to designs used for anti-counterfeiting effect pigments. This system of microlenses and color mirrors allows the user to image different colored specular highlights. An infinite number of spectral reflectance profiles can be created with these color targets and used not only colorimetrically, but also for multispectral imaging applications. The targets are not limited to the visible region; they can also be designed to work in the ultraviolet and infrared wavelength regions. This paper will describe how these targets work, the image capturing considerations (flat-fielding, white balance, etc.), and some experimental results.

Introduction

In macroscopic imaging, painted color targets are often used to calibrate and characterize cameras, scanners, and printers. For image capture under magnification, such as with a loupe or a microscope, determining the color ground truth becomes more challenging. An interference coated, color mirror target is more advantageous to use under magnification compared to a traditional painted target. Photolithographic techniques can be used to create small, and very advanced color mirror arrays so that many colors can be captured in a single image, no matter the magnification. The small arrays will have a similar reflected spectral performance when compared to a larger sample from the same fabrication cycle [1]. Photolithography is not compatible with paint. Paint consists of many sized particles that do not create homogeneous color under magnification and there can be shadowing or contamination that changes the color in localized regions. Color mirrors are fabricated under vacuum on smooth, specular surfaces reducing the probability of particulate contamination. The mirror's specular surface allows one to image their colors with only a small fraction of the light necessary compared to painted samples, allowing for shorter exposure times. Paint is susceptible to environmental conditions and will fade over time; color mirrors do not fade. Interference coating designs can create spectral reflectance profiles of any shape imaginable for not only the visible wavelength region, but also the ultraviolet and infrared regions, allowing one target to be used with a variety of camera sensors. They can also be used to create highly chromatic color mirrors. This ability expands the color gamut of a target, which is useful when imaging objects such as bird feathers or butterfly wings. Lastly, they can be used to create an ideal training target for spectral reflectance reconstruction in multispectral imaging applications reducing the sample number significantly from those using paint [2]. One issue with these color mirrors is their sensitivity to increasing incidence angle. The spectral reflectance will shift to lower wavelengths at higher incidence angles. There are design techniques that can be utilized to minimize the angular sensitivities of interference coatings [3, 4].

For transmission microscopy, an array of colored gels or interference bandpass filters has been used successfully as color targets [1, 5]. These filters are not as convenient to use in an off-axis reflection system because they are flat and specular, and these microscopy systems are typically used to image diffuse reflected color. Light sources that are placed at 45° incident to a flat, specular sample will have their light reflect off the surface at 45°, never entering the microscope lens. The key to capturing specular reflected light is to create a surface structure similar to a convex mirror that will reflect the incident light up into the microscope. The configuration described is shown in Figure 1. Here, convex microlenses reflect the specular light up into the microscope and are imaged as a *specular highlight* off of the individual microlenses.

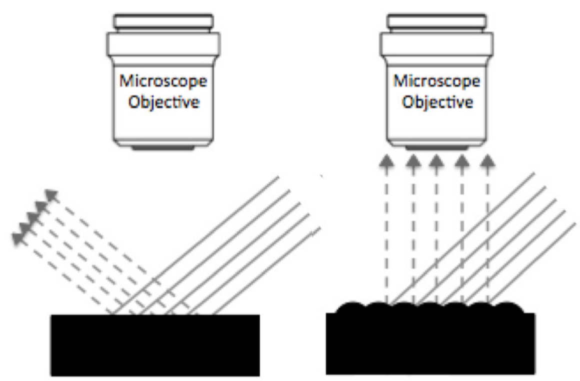

Figure 1. *Specular reflected light from a flat surface (left) does not reach the microscope when illuminated at 45°. Specular light can be reflected up into the microscope if the incident plus reflected angles total 45°. This can only happen if the sample contains convex lenses (right).*

In photography, a specular highlight can give an indication of the gloss associated with an object. It can also indicate the direction and color of the incident light source. In many imaging techniques it may be required to clip the highlight to increase the contrast of the image. Specular highlights from shiny color mirrors are not solely the color of the incident illumination. It is the combination of the spectral power distribution of the source and the spectral reflection of the color mirror. Examples of the color of both types of specular highlights is shown in Figure 2. The gum balls in Figure 2(a) are glossy, and regardless of the color of the candy shell, the color of the highlight is white. Christmas ornaments, such as those pictured in Figure 2(b), have a metallic color (either by metallic paint or powder coating). The highlights created on the surface of these color mirrors are not white unless the color of the mirror is silver; they represent the color of the light source and the spectral reflectance of the surface of the color mirror combined. Optical interference coatings give more color combinations than can be produced by any other metallic coloring process, and the reflectance profiles can be tuned for very specific applications.

(a) (b)

Figure 2. Specular highlights off of (a) glossy gum balls, and (b) metallic Christmas ornaments. The color of the specular highlight for the gum balls will be the same color as the source but for the Christmas ornaments, the color will be the combination of the spectral power distribution of the source and the reflected color of the metallic surface.

For our use of the specular highlight, it is important to prevent highlight clipping when capturing it in an image. The shapes of the specular highlights will depend on the surface structure of the illuminated microlens arrays. There will be the brightest highlight in one section of the microlens image and then the highlight will fall off depending on the shape of the lens and the distribution of light from the source. A typical microscope image for the highlight created by cylindrical microlenses illuminated by a 45° incident fiber-optic source is illustrated in Figure 3.

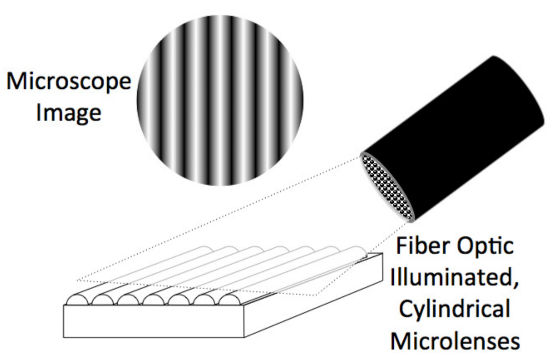

Figure 3. The light source and microlens set-up, and microscope reflected image.

Color mirrors created by optical interference coatings have the same specular behavior as their bandpass filter counterparts referenced previously. Their color is not created by a combination of absorption and scatter, as with paint, but by interference of multiple layers of thin films. There are many different interference coating design recipes that can be used when developing a color mirror: some that are all dielectric in nature, others that incorporate metal layers. The spectral reflectance of an interference coating design is based on the interference of reflected light off of each film layer interface for each wavelength of light. An interface is where the refractive index differs between two mediums or film layers. The incident light reflects off of each independent interface and changes phase. Depending on the phase, ϕ, of each reflected beam, the beams can combine completely due to constructive interference (where the phase difference, $\Delta\phi$, is a multiple of 2π), null each other out completely due to destructive interference ($\Delta\phi$ is an odd multiple of π), or create an intermediate magnitude between the two. The amount of light reflected and the phase on reflectance is controlled by the thickness of the layer and its refractive index.

The designs used for these color mirror targets are similar to some used for anti-counterfeiting effect pigments [4, 6-11]. A simple principle for reduced angle sensitivity in designs was used for all of the color mirrors: keep whatever dielectric materials used to a minimum optical thickness for a particular wavelength, shown in Equation 1, where λ is the wavelength of light, n is the refractive index of the dielectric film and d is the physical thickness. Large optical thickness and low index of refraction of a dielectric film layer are directly proportional to large angle sensitivity in optical interference coatings.

$$\lambda = nd \tag{1}$$

Sixteen color mirror designs were fabricated on a series of plastic microlens samples. These mirrors were designed to have no more than three film layers to save on production costs but still have reflected spectral shapes that covered different regions in the visible spectrum. Each color mirror was created by depositing either a single layer of titanium dioxide (TiO_2) directly on the plastic, a protective layer of silicon dioxide (SiO_2) on aluminum (Al), or three layer system of metal-dielectric-metal (opaque Al, TiO_2, and a thin, translucent layer of Inconel metal). The protective layer of SiO_2 produced reflectance similar to that of a bare metal surface. In both the single layer of TiO_2 and the three layer designs, the optical thickness of the TiO_2 determines the color reflected. The spectral reflectance profiles are shown in the Appendix and the individual layer physical thicknesses for each sample are listed in Table 1.

Experimental

A microspectrophotometer (MS) was constructed using a manual compound microscope, a Liquid Crystal Tunable Filter (LCTF), and a monochrome camera. The camera assembly was coupled into the right eyepiece opening of the microscope. The specific equipment used were a Leica GZ7 stereo microscope, a CRI Varispec LCTF, a Lumenera LW165m, 1.4 megapixel monochrome camera, and an AmScope SLR/DSLR Camera Adaptor Lens NDPL-1(2X). An incandescent, fiber-optic gooseneck source was set at 45° incidence to the sample. The magnification range that was achieved for this system was 2X-14X. All of the spectral images captured were at the highest magnification of 14X. The LCTF was tuned to 27 centroid wavelengths from 440nm to 700nm in 10nm increments. The normalized transmittance of each centroid are shown in Figure 4. The incident light on the microlens arrays was extrapolated using OptiLayer Professional [12] optical coating design software to be in the range of 20°- 60°.

Table 1: **Coating Matrix. Film physical thickness in nanometers.**

Part #	Al	SiO_2	TiO_2	Inconel	Color
1			150		Blue
2			170		Cyan
3			225		Yellow
4			280		Magenta
5	100	30			White
6	100		70	8	Sky Blue
7	100		140	8	Gold
8	100		160	8	Magenta
9	100		180	8	Violet
10	100		200	8	Blue
11	100		215	8	Cyan
12	100		235	8	Bluish-green
13	100		250	8	Yellowish-green
14	100		265	8	Yellow
15	100		280	8	Light Orange
16	100		330	8	Pink

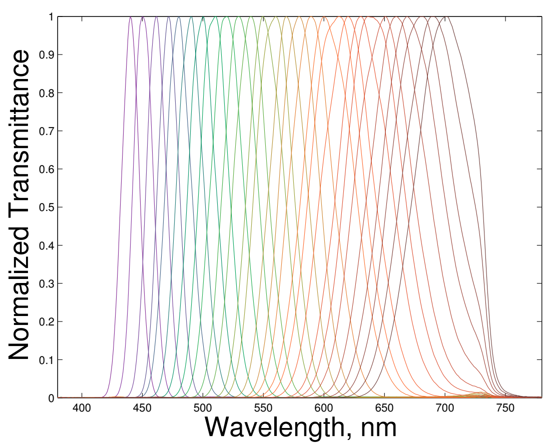

Figure 4. *Spectral transmittance of the 27 centroid wavelengths of the LCTF.*

The lens used to couple the camera assembly to the microscope eyepiece contributed to a significant amount of chromatic aberration along the outside edges of the imaging field. This lens was not specifically designed for the focal distance required for the LCTF/camera. Therefore, an image location where the chromatic aberration was at a minimum had to be determined before any imaging of the color mirrors took place. This "sweet spot" was used to compare all of the images taken using the MS.

The specular highlight samples do not create a uniformly distributed field, so flat-fielding has to rely on a uniform diffuse surface. This was achieved by using a white tile, such as one that is used to calibrate a spectrophotometer. The final tile image was the average of five separate image locations on the tile, which provided a fairly uniform white across the image. There were two separate exposure times taken, one for the white sample, and one for the Al metal color mirror (and the subsequent color mirrors). The Al represents a white metal; it would represent the "white" for the specular highlights. In order to prevent clipping of the specular highlights, one had to have a separate, shorter exposure time (only 6% of the time for the white tile). The white tile image was then used to flat-field and white balance all of the images. The difference for this example is that the flat-fielded, white balanced digital counts for the Al mirror were normalized to 0.8784 across the 27 channels, to match a spectrophotometer measurement of the specular reflected light from a flat glass sample with the same metal coating (see reflectance of sample 5 in the Appendix). Whatever normalization used for the Al mirror was subsequently used for all of the other color mirrors.

The cost to create one sample with an array of color mirrored microlenses was cost prohibitive, so each design was deposited on a separate plastic substrate. Aligning the samples rotationally such that the specular highlight was at its maximum for each sample was a difficult process and not amenable to calibrating by eye. The aluminum sample (after white balancing) was used as the baseline. The ratio of the summed image pixels using the 700nm bandpass, for the aluminum sample and each color mirror was compared to the theoretical ratio of spectral reflectance at 700nm to determine if the color mirror was aligned properly.

Once microlens alignment, flat-fielding, dark current correction, and white balance were complete, the pixels from the color mirrors were ranked based on intensity, and the top 20 pixels were averaged. This was performed because of the fall off for each specular highlight and the limited number of sweet spot image pixels. These averaged pixels represent the reflected color of the specular highlight.

A 1nm bandwidth, dual-beam Perkin-Elmer Lambda 950 spectrophotometer was used to measure the specular reflectance of the flat glass samples with the same optical coating at 20° incidence. These measurements were compared to the designs using OptiLayer Professional [12] design software for a single incidence angle and then extrapolated for the cone angle associated with the light source of the MS. These extrapolated data were reconvolved to match the bandwidth of each centroid wavelength of the LCTF.

Eight GretagMacbeth NetProfiler STF109 Color Tiles were imaged using the MS for comparison. Each tile image was an average of five image locations on the color tile, as with the white tile. The tiles were also measured using a GretagMacbeth Eye-One Spectrophotometer for spectral reflectance, and its 10nm bandwidth deconvoled to 1nm and reconvolved to match each centroid wavelength of the LCTF.

The root-mean-squared (RMS) difference of the spectral reflectance measured from the MS and the Eye-One or the OptiLayer theoretical reflectance was calculated using Equation 2 where R_{ms} is the MS measured reflectance and R_t is either the Eye-One reflectance or the theoretical reflectance.

$$RMS = \sqrt{\frac{1}{N}\frac{1}{\lambda}\sum_{j=1}^{N}\sum_{\lambda}(R_{ms,\lambda,j} - R_{t,\lambda,j})^2} \quad (2)$$

Results and Discussion

The MS measured spectral reflectance of the eight color tiles versus the Eye-One measurements are shown in the Appendix in Figure 7. The measured spectral reflectance of the color mirrors' specular highlights versus their theoretical reflectances are shown in the Appendix in Figure 8. The RMS data for each color mirror and each color tile are shown in Figure 5. It is encouraging that the average RMS data for the color mirrors is only twice that of the tiles seeing that the amount of averaged pixel data used to create the reflectance curves were different by several orders of magnitude. It is also encouraging that even with the chromatic aberrations of the microscope and camera, and the need for individual sample alignment that the spectral reflectance profile of each of the color mirrors is very close to the theoretical.

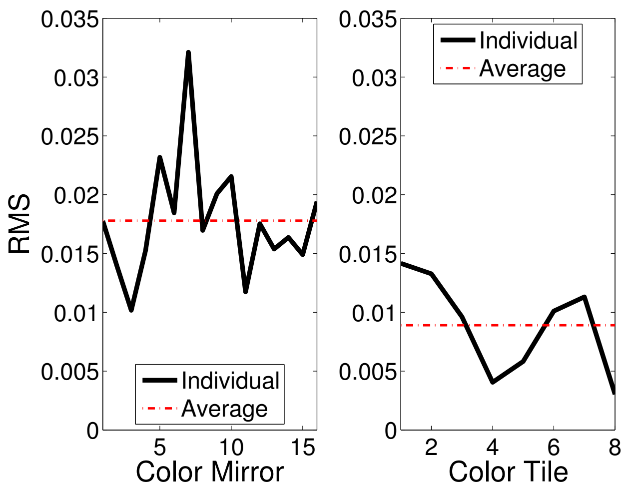

Figure 5. RMS comparison for the Color Mirrors and the Color Tiles from spectral reflectance data shown in Figures 7 and 8.

If we examine some of the wavelength regions where there were discrepancies between the measured and theoretical data we see the most difference for the 450nm - 500nm and the 600nm - 700nm wavelength regions. The reduced reflectance that appears in the 450nm - 500nm region for some of the samples may be attributed to noise created by the low light available from the incandescent source in combination with the low transmittance of the LCTF and low quantum efficiency of the sensor in the blue. The chromatic aberration is also severe for the blue and red band-pass regions. Even with a sweet spot identified within the image, there is still the potential that the aberrations blur the highlights, decreasing amount of light in the maximum pixels used in the average for the shorter and longer wavelength regions. These differences for the tiles are less severe because so many more pixels were averaged within those images.

The next steps for this research are to fabricate designs that would provide chromatic color for very narrow wavelength regions across the visible. It is difficult to isolate a single reflectance peak with the current three-layer design. Other designs that utilize thin metal-dielectric layer combinations use more layers to suppress multiple reflectance peaks and isolate a single peak [10, 11]. Recently, metal-dielectric designs have been proposed where the spectral reflectance profile resembles a theoretical "block dye" [4]. These block dye-like profiles are illustrated in Figure 6. These designs can also be extended to the ultraviolet and infrared wavelength regions as long as the dielectric materials remain transparent. These chromatic colors can be used to create an ideal training target for multispectral imaging systems. Future work should also include creating a single sample with an array of reflected colors, improving the functionality and reducing the chromatic aberrations of the MS, as well as comparing the color differences with a collimated versus a diverging light source.

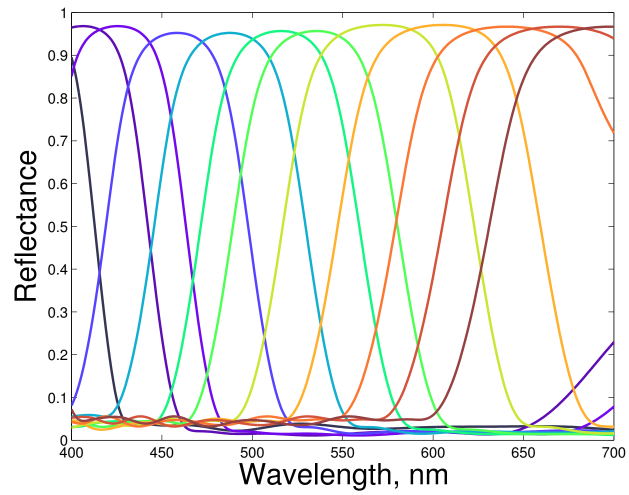

Figure 6. Spectral reflectance profiles from 12 "Block Dye" designs [4]. Each reflectance profile is an integrated reflectance curve for one specific design integrated over the incident angle range of 20° to 60°.

Conclusions

It is possible to use colored specular highlights for color targets in off-axis illumination reflectance microscopy. These specially designed targets can be fabricated with an infinite number of spectral reflectance profiles giving great flexibility to those who need reflected color ground truth in their microscopic color images.

Acknowledgement

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Author Biography

Jennifer D. T. Kruschwitz is pursuing her Ph.D. in Color Science from Rochester Institute of Technology as the Macbeth-Engel Fellow. She has received both her B.S. (1989) and M.S. (1995) in Optics from the University of Rochester. She is an Adjunct Assistant Professor in Optics for both the University of Rochester and the University of Arizona. She has worked in the field of optical interference coatings for more than 25 years and has been an independent consultant in Rochester, NY for coating design since 1998. Her main focus has been optical coating design for display, lighting, and effect pigments. She is a Senior Member of the OSA and a member of SPIE.

Roy S. Berns is the Richard S. Hunter Professor in Color Science, Appearance, and Technology of the Munsell Color Science Laboratory at Rochester Institute of Technology, USA. He received B.S. and M.S. degrees in Textiles from the University of California at Davis and a Ph.D. degree in Chemistry from Rensselaer Polytechnic Institute (RPI). Berns has received scientific achievement awards for the Inter-Society Color Council, the Society of Imaging Science and Technology, the Colour Group of Great Britain, and the International Association of Colour. He is the author of the third edition of "Billmeyer and Saltzman's Principles of Color Technology".

Appendix

The following are the spectral reflectance data for the eight color tiles (Figure 7) and 16 color mirrors (Figure 8).

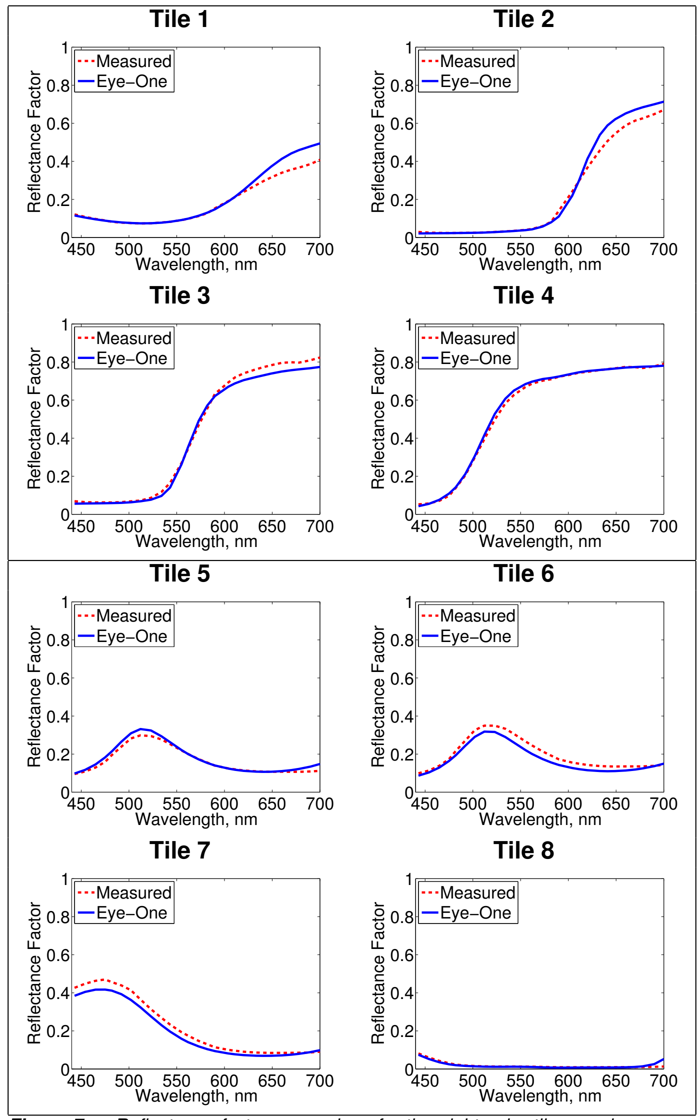

Figure 7. *Reflectance factor comparison for the eight color tile samples between the Eye-One (solid blue) and MS (dashed red), both with matching bandwidth.*

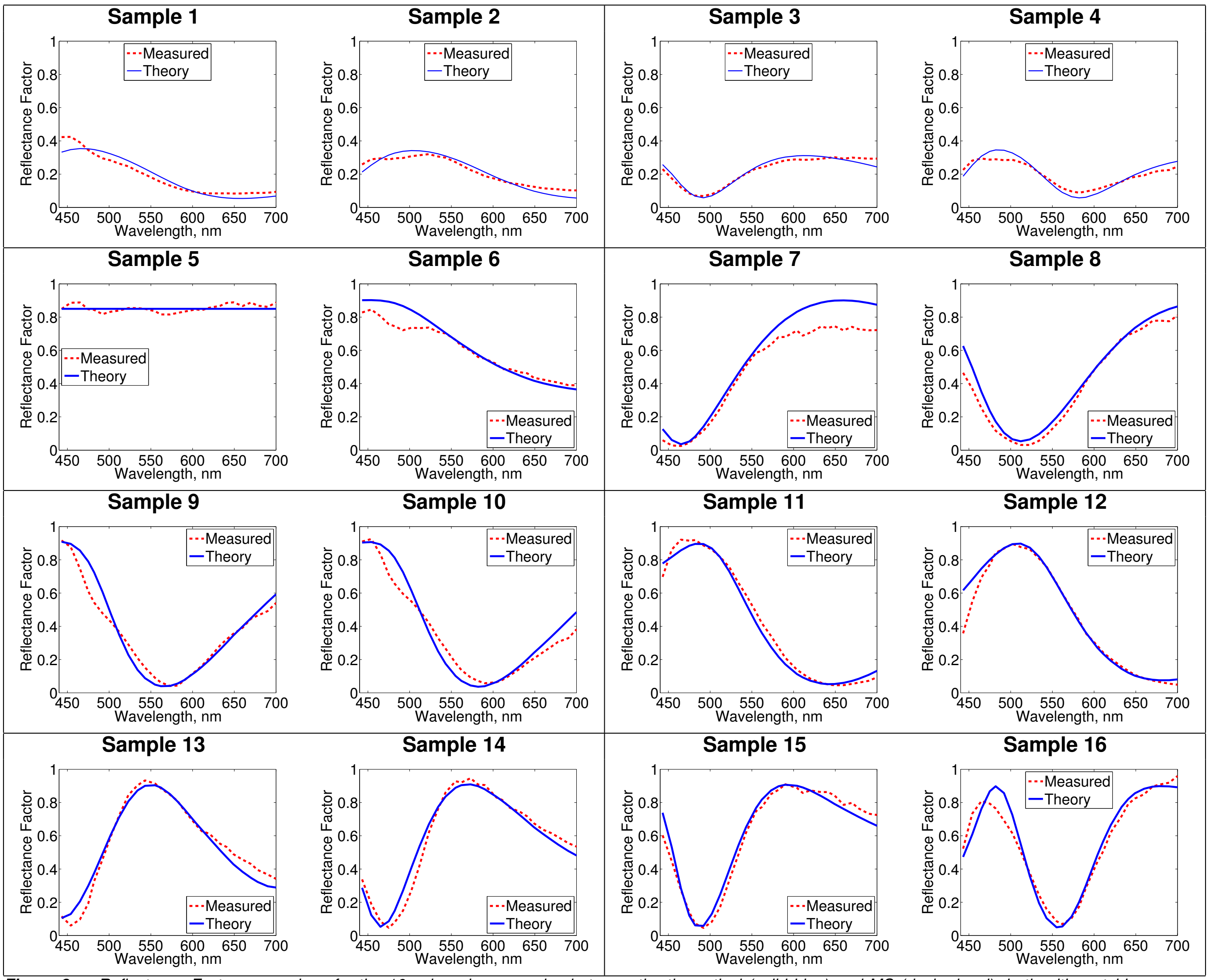

Figure 8. *Reflectance Factor comparison for the 16 color mirror samples between the theoretical (solid blue) and MS (dashed red), both with matching bandwidth.*