1 Introduction

The source of contrast in reflectance confocal microscopes is refractive index variation within the sample. Such variations result from many cellular structures, ranging from protein granules in intracellular organelles to the epithelial-stromal interface. These refractive index variations contain spatial frequency variations from less than 1 line pairs per millimeter (lp/mm) to over 1000 lp/mm. Because the information content exceeds the diffraction limit of near-infrared optical systems, the appearance of images acquired from these instruments depends on the modulation transfer function (MTF) of the instrument.

Presently, multiple-center clinical trials are under way to acquire in vivo images from pigmented lesions using reflectance confocal microscopy. These images are correlated to histological slides of biopsy specimens from the same lesion to develop diagnostic criteria. The diagnostic criteria will be used to test the sensitivity and specificity of reflectance confocal microscopy in the management of pigmented lesions. Because the diagnostic criteria are being developed in a consensus process using confocal images acquired by different imaging systems, it is important to consider the MTF of each system when evaluating the images. Therefore, a simple to use method to measure the MTF of each system used in the study is required. Such a method also provides a useful standard for the Clinical Laboratory Improvement Act (CLIA) (http://www.cms.hhs.gov/clia/) testing of such systems to ensure operation within its desired resolution specification.

One traditional way to measure MTF is to use a confocal test specimen in which patterns of various spatial frequencies are etched on a film. The contrast at each frequency is measured to obtain the MTF. This method has several disadvantages. The specimen is challenging and expensive to manufacture. Only the contrast at the frequency patterned on the substrate can be measured. To get a continuous MTF curve, multiple measurements are required, which is time-consuming. Additionally, for each measurement, the pattern with desired frequency has to be centered in the field of view because space invariant imaging cannot be assumed. Another well-established method to measure the point spread function (PSF) uses polystyrene microspheres with dimensions much less than the lateral PSF. The MTF can be calculated by Fourier transforming the PSF. However, this requires oversampling the microsphere image. For systems that normally operate at the Nyquist limit, oversampling requires that the resolution be measured in a slow-scanning rate mode or fast-sample mode. This implies that the MTF is measured in a system configuration that is different from what is used in patient imaging. Furthermore, many confocal microscope systems used in clinical trials, which are designed to acquire real-time images, often cannot be adjusted to such a slow-scanning rate or fast-sample mode.
The ISO-12233 standard\textsuperscript{4} has been established to measure the spatial frequency response (one-dimensional MTF) of an electronic imaging system that uses a regularly spaced imaging sensor. The MTF is calculated from the edge response function of a slanted line in a test target. When the slanted straight edge of the target is imaged by a regularly spaced sensor array, the image of the edge moves in a predictable manner from row to row across the pixels and thus allows for an oversampled representation of the edge response for detector spacing at the Nyquist limit by accumulating data over multiple rows. Algorithms for deriving the MTF from the slanted edge images are available from the International Imaging Industry Web site (http://www.i3a.org/downloads.iso.html). However, the ISO-12233 target cannot be used with scanning spot imaging system because scan errors remove the predictable row-by-row offset of the slanted edge. Fortunately, the asynchronous pixel clock inherent to many confocal scanning systems induces a subpixel jitter in the pixel position. This jitter adds a variable subpixel offset to each row in the image and varies the digitized intensity near the location of the physical edge. Taking advantage of this, we provide a simple to use technique similar to ISO-12233 to measure the one-dimensional MTF of a scanning imaging system with a single acquired image. Using this technique, we were able to measure and compare the MTF of three reflectance confocal microscopes by acquiring a single image of an edge target from each system.

2 Method

2.1 Instrumentation

Three Lucid VivaScope\textsuperscript{TM} reflectance confocal microscopes (Lucid Inc., Henrietta, New York, http://www.lucid-tech.com) were used in these measurements: a production VivaScope 1000 and two different VivaScope laboratory prototypes. Of the microscopes tested, only the VivaScope 1000 was being used in clinical testing. The other systems used were imaging prototypes. The goal of the measurements was to test the accuracy and precision of the algorithm to compare the imaging performance of different microscopes. Once validated, this technique could be used to compare the various instruments used in multicenter trials.

VivaScopes are Food and Drug Administration (FDA)-cleared laser-scanning reflectance confocal microscopes equipped with either a 30\(\times\)0.9-numerical aperture (NA) LOMO (St. Petersburg, Russia) or a 30\(\times\)0.9-NA ASE Optics (Rochester, New York) water immersion objective. The system details have been described previously.\textsuperscript{2} A schematic of the system is shown in Fig. 1. An 830-nm laser illumination beam is scanned by polygon and galvanometer mirrors and relayed into the water immersion microscope objective that focuses the beam into the patient. The acquired image is nominally parallel to the tissue interface. The field of view is 0.5\(\times\)0.375 mm. The depth of the image plane is adjusted by moving the microscope objective relative to the tissue surface using a 1-\(\mu\)m resolution micrometer. Light reflected from the tissue is collected by the objective, descanned, and focused onto a five-resolution element pinhole. Light passed through the pinhole illuminates a single-element silicon avalanche photodiode. The detected optical signal is digitized by an 8-bit frame grabber and displayed on a computer monitor with 640-\(\times\)480-pixel resolution. The target used to measure the lateral resolution was a chrome-on-glass stripe 55-\(\mu\)m wide. An image of the target is shown in Fig. 1.

2.2 Pixel Clock Asynchronism and Galvo Locked Scanning Mode

As shown in Fig. 1, a 36-facet polygon provides a “fast scan” of the point illumination across the lateral extent of the sample. A galvo mirror sweeps the “fast scan” line across the sample vertically forming the “slow scan” raster. An auxiliary laser reflects off each polygon facet and strikes a split detector to provide a “start of scan” (SOS or HSYNC) signal for each fast scan line of the image. As each HSYNC signal is asserted, an image acquisition system (National Instruments, Austin, Texas) digitizes the detector signal at approximately 5 MHz using an independent asynchronous pixel clock (Fig. 2). Given this sampling rate and scan speed, there will be 2 to 3 pixels covering the optical transition across the edge [Fig. 3(c)]. These data points are insufficient to calculate a smooth and accurate line spread function (LSF) and MTF.

As shown in Fig. 2, the HSYNC signal and pixel clock is not synchronized and the time delay between these two clocks varies by row. This asynchronous pixel clock adds a random
Figure 3 Algorithm illustration: (a) Image of the test target at normal scan mode. (b) Image of the test target at galvo locked mode. (c) Edge response from two different lines sampled by the pixel clock before and after dejittering. Note the offset is mostly reduced after dejittering. Inset shows pixel jitter along the physical edge. (d) An oversampled ESF combined from all dejittered lines. (e) LSF calculated from ESF. (f) One-dimensional MTF calculated from LSF.

offset within 1 pixel to each line of the image. Figure 3(a) shows an image of our test target at normal scanning mode (galvo unlocked). A strip with sharp edges can be identified in the center. This strip is a line of reflectance of approximately 48% (chrome-water reflection at 785 nm) on a background of 0.4% (water-glass reflection). Comparing the digitized intensity between successive pixel rows near the physical edge, the pixel jitter can be observed [Fig. 3(c) inset]. Finding the ac-
tual offset of each row and removing such jitter will provide
an oversampled edge to calculate the LSF and one-
dimensional MTF. At normal scanning mode, it is difficult to
find those accurate offsets between rows. A small slant of the
physical edge will overwhelm the pixel jitter. Image intensity
along the edge is space variant [Fig. 3(a)] due to the field
curvature of the given imaging system. Therefore, running
the microscope at galvo locked mode is required. When imaging
at galvo locked mode, the galvo (slow scan) is turned off and
the imaging laser beam is scanned repeatedly over the same
lateral line across the object. The acquired image [Fig. 3(b)]
consists of duplications of the central line of the test target
when imaged at galvo unlocked mode [Fig. 3(a)]. Because a
single line is imaged instead of the whole field, the vertical
extent of the image is space invariant and the influence by a
slanted object edge is avoided. The major difference between
lines can be attributed to the scan error by the asynchronous
pixel clock.

2.3 Dejitter Using Fourier Shift Theorem

We use a Fourier method to calculate the offset of each line
caused by the asynchronous pixel clock. According to the
Fourier shift theorem, a delay in the spatial or time domain
corresponds to a linear phase term in the frequency domain
(1). Using the first line of the image as a reference \( I_{ref} \), the
offset of the \( j \)th line \( \Delta_j \) relative to the reference can be
calculated from the phase difference \( PD \) in the frequency
\( f \) domain Eq. (2). A linear fit between \( f \) and \( PD \) to calculate
\( \Delta_j \) is required to reduce error by noise. The jitter is then
removed by shifting each line in the amount of \( -\Delta_j \) [Fig.
3(c)]. Because each offset is a subpixel variable, an over-
sampled edge is derived by accumulating all the lines in a
single image after dejitter [Fig. 3(d)]

\[
FT[I_j(x + \Delta_j)] = \exp[-j(2\pi\Delta_j)f]FT[I_{ref}(x)],
\]

\[
PD = \text{Phase}\left(\frac{FT[I_j(x + \Delta_j)]}{FT[I_{ref}(x)]}\right) = (-2\pi\Delta_j)f.
\]

2.4 Calculation of Edge Spread Function, LSF, and MTF

The algorithm to calculate the edge spread function (ESF),
LSF, and MTF [Figs. 3(e) and 3(f)] is similar to ISO-12233.
The oversampled data points [small dots in Fig. 3(d)] are
binned by one-fourth the pixel width and averaged [large dots
in Fig. 3(d)]. A continuous ESF is formed from these 4×
oversampled data points. The ESF, which contains resolution
and MTF information similar to the PSF, is the imaging sys-
tem response to the real physical edge on the target. Once the
ESF is derived, the LSF can be calculated by making a two-
point derivative of the ESF. One-dimensional MTF is a
normalized fast Fourier transform of this LSF. Additionally,
the full-width half maximum (FWHM) of the LSF is used as
a measure of the lateral resolution. A MATLAB program was
developed to measure the MTF and lateral resolution from a
single image with a sharp straight edge.

2.5 Validation

An oversampled edge response, which is key to calculating
the lateral resolution and MTF, can also be acquired using a
fast oscilloscope (Tektronix, TDS5000B, Richardson, Texas)
directly connecting it to the pinhole detector. At the sam-
pling rate of 4 ns, the oscilloscope can oversample the edge
50 times faster than the 5-MHz pixel clock. Those 50× over-
sampled data points are further binned down to 4 times the
pixel rate, which matches the oversampling rate used in our
algorithm under test. Then, the lateral resolution and MTF
can be derived the same way from these oversampled data pro-
vided by the oscilloscope. We use this alternative method to
validate the results calculated from our dejitter algorithm. It
is important to note, however, that this oversampled edge re-
sponse by the oscilloscope is from one line scan and thus
from one facet scan of the polygon. Our dejitter algorithm, on
the other hand, oversamples by phase-shifting multiple line
scans, or equivalently, multiple facet scans.

3 Results

3.1 MTF Measurement of Different Imaging Systems

We measured the MTFs of three different models (prototypes)
of VivaScope confocal imaging systems (Fig. 4). As expected,
the MTF of each system has its own signature, even if those
systems were built using similar optical and electronic de-
signs. The MTF differences influence the image appearance
and may bias the image interpretation. Therefore, it is essen-
tial to consider the specific MTF of each system when evalu-
ating images, especially when clinicians try to reach unbiased
diagnosis consensus based on images acquired from different
systems. To examine repeatability, we performed six measure-
mants on the multiwavelength VivaScope prototype (solid line

![Fig. 4 MTFs of three confocal imaging systems derived from over-
sampled edge responses. (a) Multiwavelength VivaScope prototype
using a 30×0.9-NA LOMO objective and 785-nm illumination
(solid), (b) VivaScope 1500 prototype located at Lucid, Inc., using a
30×0.9-NA ASE objective and 830-nm illumination (dash dot), (c)
VivaScope 1000 located at Memorial Sloan-Kettering Cancer Center
using a 30×0.9-NA LOMO objective and 830-nm illumination
in Fig. 4) with various time intervals from seconds to minutes. Particularly, the spatial frequency at 10% visibility (contrast) is an important specification of the MTF. Results show the average spatial frequency at 10% visibility is 950 lp/mm, and the standard deviation is 9.8 lp/mm. Considering this significant order of magnitude difference, the measurement variation within a system can be neglected. It has to be mentioned here that although the ideal Nyquist frequency of a 0.9-NA optical system is about 1200 lp/mm, real systems may not achieve that due to insufficient electronic sampling. The lateral Nyquist frequency of tested VivaScope systems is 640 lp/mm, given the pixel resolution (640 × 480) and field of view (0.5 × 0.375 mm). The frequency we measured at 10% contrast, which is higher than the system Nyquist frequency, is derived from the oversampled edge response and represents the actual optical frequency response of the system.

### 3.2 LSF and Lateral Resolution Measurement

Figure 5 shows the LSF of one tested system (a in Fig. 4). The lateral resolutions of the three systems, which is measured as the FWHM of the LSF, are 0.88, 0.83, and 0.84 μm (from a to c as in Fig. 4). The same repeatability test of system (a) as mentioned in Sec. 3.1 shows the standard deviation of six lateral resolution measurements is 0.037 μm. The difference between the three systems is also small (less than 0.1 μm), which implies the MTF is more specific to characterize an imaging system than resolution. Ideally, for a confocal system with 785-nm illumination using a 0.9-NA water immersed objective, the lateral resolution is about 0.44 μm. The actual resolutions are much larger in those three systems because of residual system aberrations in the relay optics and objective lens and the large five-resolution element pinhole used to increase signal-to-noise ratio.5,6

### 3.3 Validation

As mentioned previously, the lateral resolution and MTF can be derived in a similar fashion from an edge response oversampled by the oscilloscope. We use this method to validate the results calculated by our dejitter algorithm. Figure 6 shows good agreement in the shapes of the MTF curves calculated from the two different methods. At 0.9-NA setting, the spatial frequency difference at 10% visibility (55 lp/mm) is only about 9% of Nyquist frequency of the system (640 lp/mm). The lateral resolution difference is less than 0.05 μm. A small discrepancy exists between MTF curves from two different methods. To test whether this discrepancy is systematic at different instrument configurations, we lowered the objective NA of the testing prototype to 0.35. As expected, the MTF using 0.35-NA objective is significantly narrower than using the 0.9-NA objective. However, the discrepancy between two methods remains consistent and small (Fig. 6). At both NA settings, the MTFs calculated from the edge response given by the oscilloscope are slightly broader than that of the dejitter algorithm, especially at high spatial frequency. In the dejitter algorithm, the oversampled edge response is acquired by accumulating all the lines in a single image. Intensity noise between these lines adds some errors to the offsets found by the Fourier method, which could narrow down the MTF and reduce the lateral resolution. The oscilloscope, however, can oversample an edge from a single image line so the results are less affected by intensity noise. The much higher sampling rate of the oscilloscope (250 MHz) may also explain the broader MTF at high spatial frequency. However, the limitation of using an oscilloscope is also obvious. Because one acquisition of an edge response by the oscilloscope only covers a single line scanned by one facet, to fully describe the system multiple measurements from different facets are required for an average, which is time-consuming. Users also may not have the access to reconnect the detectors to an oscilloscope. Furthermore, the availability of a fast digital oscilloscope at a clinical trial site can itself be a problem.

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**Fig. 5** LSF of the multiwavelength VivaScope prototype [(a) in Fig. 4].

**Fig. 6** MTFs of the multiwavelength VivaScope prototype at two different NAs (0.9 and 0.35) calculated from edge response oversampled by two different methods: dejitter algorithm [solid (a) for 0.9 NA, solid (b) for 0.35 NA], oscilloscope [dash-dotted line for 0.9 NA, dashed line for 0.35 NA]. Because oscilloscope only scans a line for one measurement, the MTF presented here is an average of 10 different measurements to better describe the whole system.
4 Conclusions

A fast, simple, and repeatable technique is presented to measure the MTF and lateral resolution of a scanning spot microscope with asynchronous pixel clock. It may also be used with scanning systems with optically derived synchronous clocks. In these systems, a user could add a jitter circuit in the pixel clock between the scanner and frame acquisition unit or substitute an asynchronous clock running at the nominal pixel rate. The inherent jitter of an asynchronous pixel clock allows for the derivation of an oversampled edge response function from a single image of a strip object. From the edge response function, the MTF can be easily calculated using standard numerical techniques.

MTF is a common metric of image quality giving quantitative measure of image contrast. The ability to calculate the MTF of each instrument assists in correlating images at different clinical sites and may be the basis for CLIA certification of clinical instruments.

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References