Real-Time Wavefront Coded Microscopy

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Abstract

Real-time stand-alone Wavefront Coded processors for two-dimensional filtering have recently been generated using current state-of-the-art silicon-based processing hardware on field programmable gate arrays (FPGAs). Processing speeds of up to 30 billion operations per second have been demonstrated on a one million-gate FPGA device. This represents the ability to process VGA-sized Wavefront Coded microscope images at over 100 frames per second.

Introduction

Biological, metallurgical, and machine-vision microscope systems rely on high magnification imaging systems for tasks ranging from medical slide scanning to real-time manufacturing control. In many of these processes the object under investigation is usually moving or cannot be placed with precise accuracy in the field of regard. Such cases demand a high quality imaging response from the optics over a very broad region in space. The ability of high-resolution objectives to also have a large depth of focus is limited by traditional lens design techniques and available materials. Wavefront Coded Microscopy brings a new paradigm to the microscope user by enabling large depth of focuss to be obtained without reducing aperture sizes or requiring expensive optical materials. By combining aspheric optics with digital signal processing, high magnification and high resolution images can be obtained in real-time for a large depth of focus at a reasonable cost through Wavefront Coding.

A modest data rate for microscope images is approximately 1k x 1k pixels per frame at 30 frames per second which generates 30 million pixels per second. A non-separable Wavefront Coded reconstruction kernel might contain 32 x 32 elements, or 1024 coefficients. This kernel requires 1k computations per pixel, and the system produces 30M pixels per second, thus it requires 30 billion multiply-adds per second for reconstruction. In this work, a hardware processor based on a 1 Million gate FPGA has been utilized to implement an example system and provide Wavefront Coded extended depth of focus microscope images in real-time.

High magnification imaging systems are used throughout biological and metallurgical research as well as in real-time manufacturing control. Typically the object being observed is moving or must pass through the viewing area in a limited amount of time. Due to such throughput constraints, processing time for image formation must be minimal. In this paper we demonstrate a real-time processor that enables a larger depth of focus to be obtained through Wavefront Coding.

Wavefront Coding Primer

Wavefront Coding is based on sound practices from linear systems, information theory, and classical lens design.[1] Wavefront Coding systems are non-traditional optical systems that produce images that are insensitive to system error and aberrations that typically produce blurred images. In linear systems jargon, such systems are "invariant" to system errors.[2] Information theory dictates that an invariant system maximizes image information. In the strict terms of classical lens design, such a system is simply not possible since use of digital processing as part of the image formation process is not considered! Through the revolutionary techniques of Wavefront Coding, such systems have been proven practical in applications ranging from microscopes to telescopes.

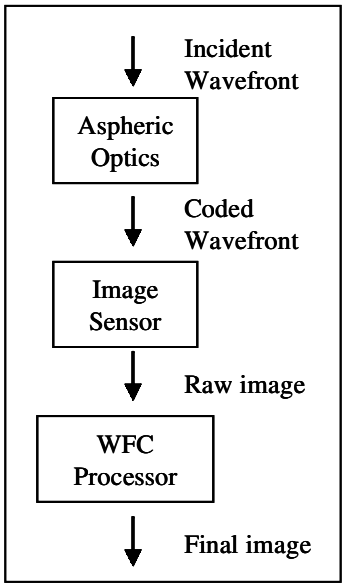

Figure 1. System block diagram. Specialized optics produce images that are insensitive to misfocus like aberrations. A blurred "raw" image is digitized at the detector. Processing of the digital blurred image produces a sharp final image free of aberrations.

A block diagram of a general Wavefront Coding imaging system is shown in Figure 1. The optics in a Wavefront Coding system are specialized aspheres which act to make the sampled images insensitive to focus-like aberrations such as misfocus, chromatic aberration, astigmatism, field curvature, assembly related misfocus and temperature related misfocus. The sampled image is then processed with object-independent signal processing to produce a sharp image. This processed image can be used for display or for specific image analysis.

The assortment of classical aberrations that can be controlled with Wavefront Coding leads to the concept of an "aberration budget". With an aberration budget the proportion and amounts of misfocus aberrations do not need to be known in advance. The aberrations can be fixed or dynamic. It is the maximum total amount of misfocus aberrations that are specified as a part of the system. It is only the cumulative total of the misfocus aberrations that must remain below a system dependent value for high quality imaging.

Real images provide a good example of invariance to misfocus in an actual Wavefront Coding imaging system, as shown in Figures 2 and 3. Figure 2 shows experimental images of point objects or point spread functions (PSFs) at two focus positions, in focus and out of focus for a traditional and Wavefront Coded microscope.

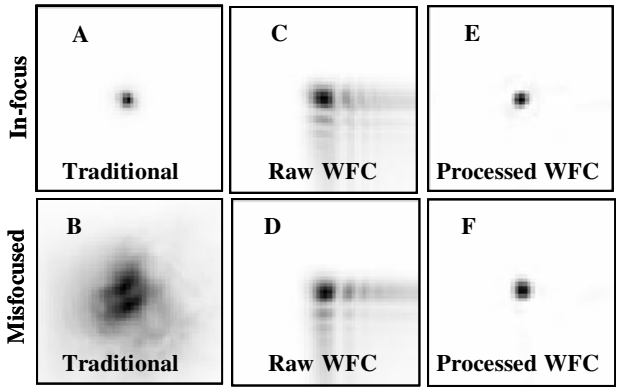

Figure 2. Imaging of point objects. Traditional images of in-focus point objects form clear images (A). When the point object is far from the best-focus object plane, a blurred image results (B). Images of a point object from Wavefront Coded optics before signal processing have a non-traditional profile that is insensitive to misfocus (C and D). After misfocus-independent signal processing the Wavefront Coded image of the in-focus and out-of-focus point is essentially the same as the in-focus traditional image. The processed Wavefront Coded image is independent of focus (E and F). All images have the same scale.

Figure 3 shows modulation transfer functions (MTFs) as a function of misfocus related to the PSFs from Figure 2. The PSFs and MTFs from traditional systems are very sensitive to misfocus. The PSFs and MTFs from a comparison Wavefront Coded system show essentially no change with misfocus. A misfocus independent digital filtering process used to restore the Wavefront Coded PSFs and MTFs to be similar to that of the diffraction-limited in-focus system

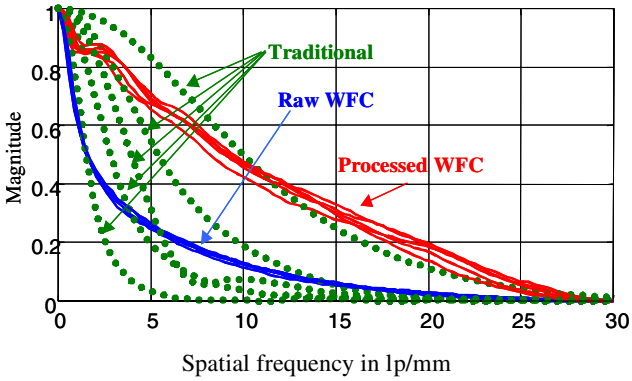

Figure 3. MTFs as a function of misfocus. Traditional system drastically loses spatial resolution with misfocus. Wavefront Coded MTFs before signal processing are lower than the in-focus traditional system but essentially constant with misfocus. After signal processing all Wavefront Coded MTFs are very similar to the in-focus one.

In Figure 3, notice that the MTFs of the Wavefront Coded imaging system before signal processing, while being constant with misfocus, are also lower than the traditional in-focus MTF. Signal processing applies a gain and phase to each spatial frequency to produce the final MTFs. This amplification affects both the deterministic components of the image and the additive noise. In order to reduce the amount of noise amplification, the MTFs before signal processing are designed to be as high as theoretically possible.

Real-Time WFC Processing

The algorithms developed were shown to be scalable to process non-separable kernels of any size and images of any size. In practice the size of filtering kernels allowed is limited to the resources in the selected FPGA, and also the design tool capability for synthesizing a workable core within a reasonable amount of time. Speed limitations on the FPGA devices (typically only hundreds of MHz) limit both the ultimate size of the designs and throughput of the device.

The basic design concept for a Wavefront Coded reconstruction core consists of a series of buffers and single-element taps. The buffers consist of block RAM elements which delay the pixels appropriately for the image size and kernel size. The tap structures perform scaling on their respective input pixel values and an adder and scaling provides the final tap output. Figure 4 shows a flow diagram of the basic algorithm.

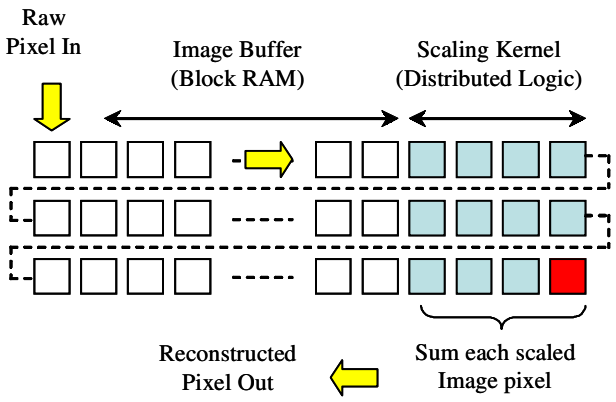

Figure 4. Block diagram of the basic convolution algorithm. Buffers are used for delay-line structures. The taps perform scaling on the input pixel values and an adder provides the final tap output.

The design is scalable for a variety of reconstruction kernels and image sizes. Larger image sizes increase the requirements for delay lines and hence can increase the core size significantly. Kernel sizes can also scale the design size, so compact kernels are desired in hardware processing as well as software processing. For very large image sizes, off-chip RAM could be utilized rather than internal RAM blocks for performing the delay line.

Some Wavefront Coded optical systems can be processed in a separable fashion, where the columns of the image are filtered independently of the rows. Such filtering is more efficient mathematically than two-dimensional, or non-separable, spatial filtering. A rectangularly separable design similar to that described above would contain 32+32=64 coefficients and require 640 million MACs per second. Both separable and non-separable processing has use in WFC reconstruction and both systems are useful for microscopy. The processing must also be scalable in image size, kernel size, and frame rate, as future WFC microscope systems for biological and medical use can approach 4k x 4k sensors operating at 25 to hundreds of frames per second.

Example Results

Images were acquired using a Nikon TE300 inverted microscope with a 60XA, 1.4 numerical aperture objective (oil immersion). A processing core for a 17x17 reconstruction kernel with a 1k x 1k image size was generated. The core consumed nearly 100% of the resources on a Xilinx Virtex-2 1000 device, a million-gate part. A graphic of the reconstruction kernel is shown in Figure 5. The processing core was loaded onto the FPGA and integrated with a Uniq-UP1830 camera that generates 1024x1024 sized images at 30 frames/second, which corresponds to a 45MHz pixel clock.

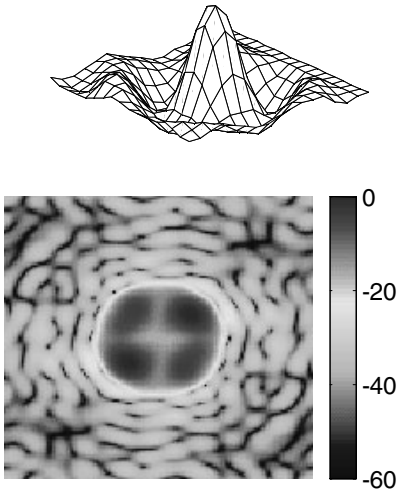

Figure 5. An example 17x17 reconstruction kernel and its frequency response, shown in dB.

Diatoms

Images of a roughly spherical diatom were obtained from the microscope and processed in real-time using the FPGA. This particular configuration provides a depth of focus increase of 2 to 4 times the original depth.

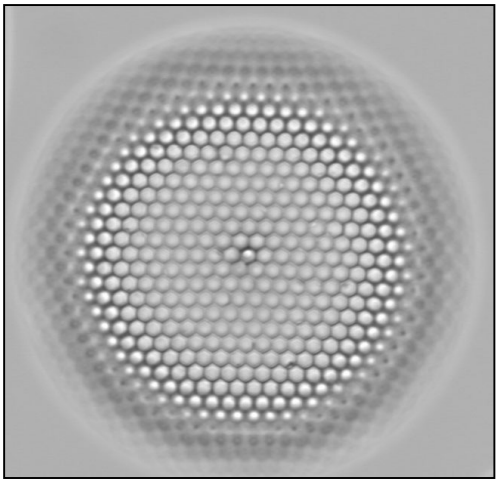

Figure 6. Traditional microscope image. Only an annular region with a thickness of about 1/4 the radius of the object is in focus. The in-focus annulus is the ring of highest contrast, roughly centered between the middle of the object and its edge.

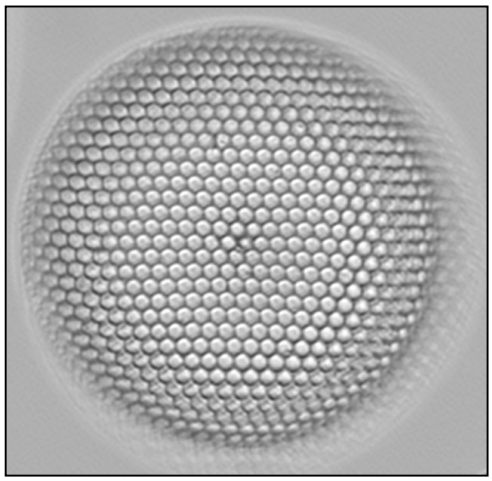

Figure 7. Wavefront Coded microscope image, reconstructed at 30 frames/second with a 17x17 sized kernel. Note the clear depth of focus extension in the Wavefront Coded image. The depth extension is approximately 2 to 4 times.

Pap Smear

Traditional and Wavefront Coded images of a Papanicolaou smear are shown in Figures 8 and 9. Real-time rendering of high-depth of focus images for automated analysis and screening provides more information without varying focus or stepping through focus increments.

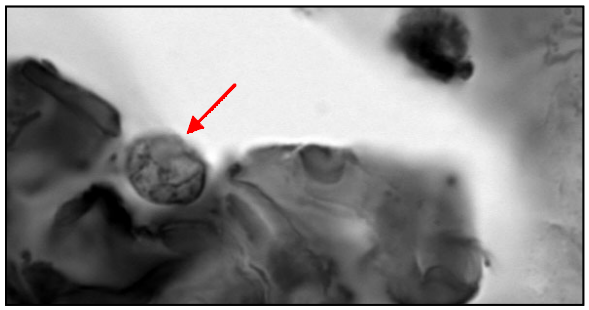

Figure 8. Traditional microscope image of a Papanicolaou smear. Note the lack of structure in the tissues that are beyond focus.

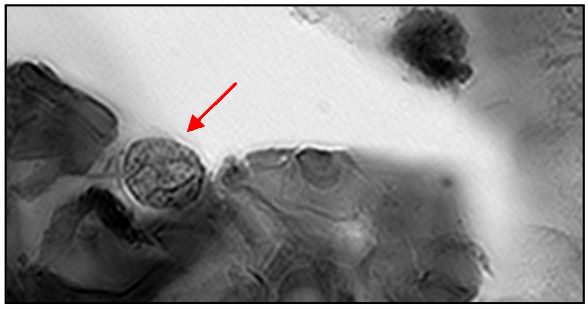

Figure 9. Wavefront Coded image of a Papanicolaou smear. Note the detail in the taller cellular structures. This example appears to have approximately a 2-times depth of focus.

Pond Organisims

Traditional and Wavefront Coded images of simple pond water are compared in a time-lapse sequence. The sequence of images in Figures 10 and 11 were obtained at 0.2 second intervals, or 5 frames/second for each sequence. The oblong organism is rotating and moving rapidly in and out of the plane of focus.

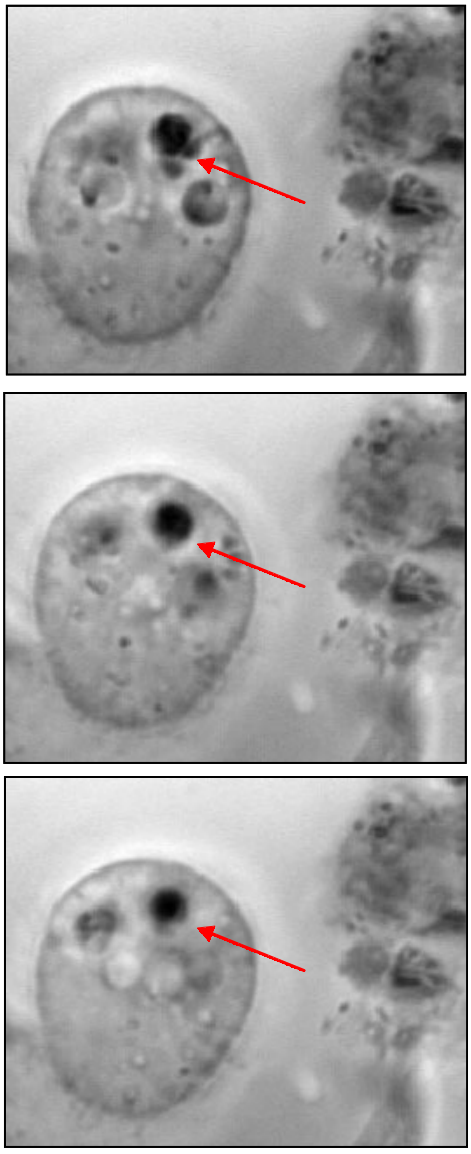

Figure 10. Traditional microscope sequence of a rotating pond organism Note the variation in features and position with each image as the organism moves in the water.

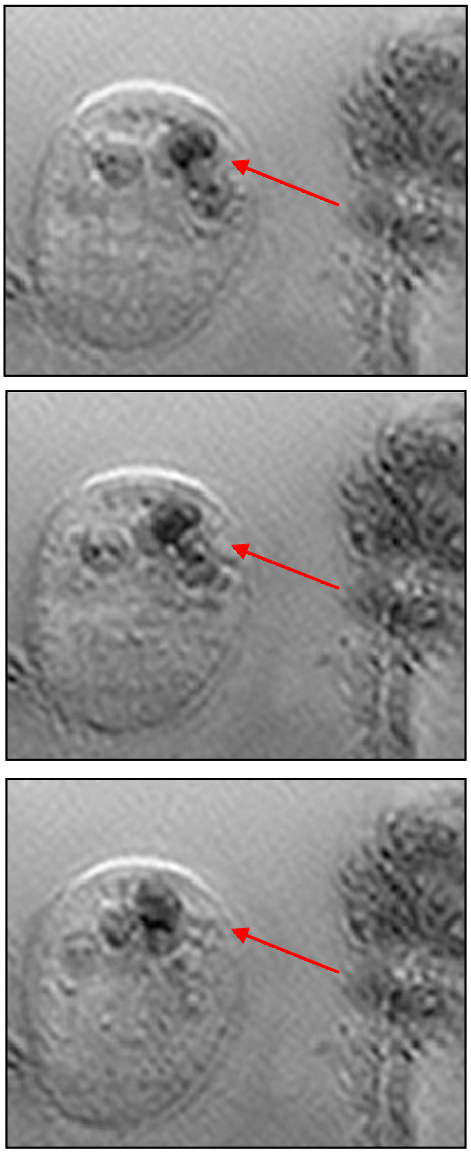

Figure 11. Wavefront Coded microscope sequence of a moving pond organism Note the increased depth of focus as compared to the traditional image sequence.

The organism in Figure 11 shows several structures in the darker region, as opposed to the single dark smooth structure indicated in Figure 10. Furthermore, the interaction of the potential multiple bodies is shown through variations in Figure 11, whereas Figure 10 only shows a single dark spot in all three images.

The ability to monitor living organisms with extended depth of focus can provide insight not previously available since more structures can be seen simultaneously. Faster recording times for larger volumes could lengthen the lifetime of living organisms under such intense scrutiny.

Conclusion

Real-time Wavefront Coded microscopes can usefully extend the depth of focus of traditional microscope images. Utilizing specialized cores with FPGA processors, images can be reconstructed at billions of pixels per second, allowing easy user interaction, high-speed screening, or manufacturing control systems applications through real-time reconstructions.

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References

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2. E. R. Dowski, “An Information Theory Approach to Incoherent Information Processing Systems”, Signal Recovery and Synthesis V, OSA Technical Digest Series, 106-108, 1995.

Biography

Greg Johnson received his Master’s degree in electrical engineering at the University of Michigan, Ann Arbor, MI in 1996 and his PhD degree in signal processing at the University of Colorado, Boulder, CO. He is currently the Research Director for CDM Optics, Inc., of Boulder, Colorado, which designs and manufactures Wavefront Coded imaging systems. His current research interests include nonlinear image processing, Wavefront Coded systems design and optimization, and design methods using multi-dimensional signal decomposition.