NIR Multimodal Microscope with In-Situ Pulse Characterization Tools

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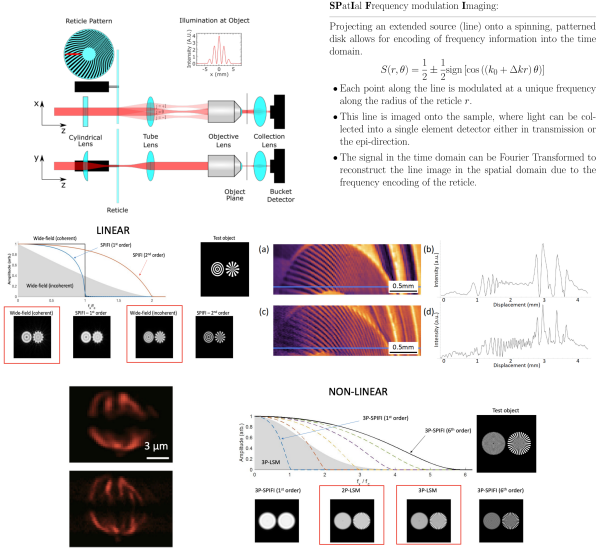
Abstract

Our **SP**at**I**al **F**requency modulation **I**maging system (**SPIFI**) employs two stages of modulation: the first stage is the Wavelength Domain (WD) SPIFI unit, and the second stage is the Spatial Domain (SD) SPIFI unit. The WD-SPIFI can be used to measure the amplitude and phase of the femtosecond excitation pulse at the focal plane of the imaging platform. Further, the WD-SPIFI stage enables facile, real-time correction of group-delay dispersion (GDD) which can be residual from the laser or from the glass in the system. Significantly, WD-SPIFI has this GDD control built-in, requiring no additional components. Notably the WD-SPIFI stage can also used for linear imaging, enabling fiber optic delivery of the SPIFI signal for the first time. Next, using only the SD-SPIFI stage, the frequency support and cut-off frequency limits are extended in linear and nonlinear imaging modalities (including SHG and THG, and in multiphoton excitation fluorescence imaging.) This enables enhanced resolution capability. Finally, by operating the WD-SPIFI and SD-SPIFI stages synchronously, linear excitation, two-dimensional, single element detection imaging with enhanced resolution capability is possible.

SPIFI Overview



SPat**I**al **F**requency modulation **I**maging:

Projecting an extended source (line) onto a spinning, patterned disk allows for encoding of frequency information into the time domain.

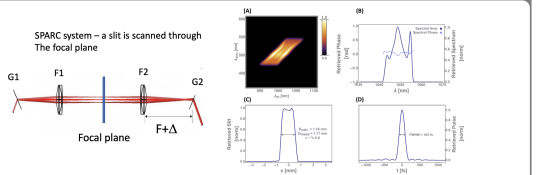
$$S(r,\theta) = \frac{1}{2} + \frac{1}{2}\text{sign}\left[\cos\left((k_0 + \Delta kr)\,\theta\right)\right]$$

- Each point along the line is modulated at a unique frequency along the radius of the reticle r.
- This line is imaged onto the sample, where light can be collected into a single element detector either in transmission or the epi-direction.
- The signal in the time domain can be Fourier Transformed to reconstruct the line image in the spatial domain due to the frequency encoding of the reticle.

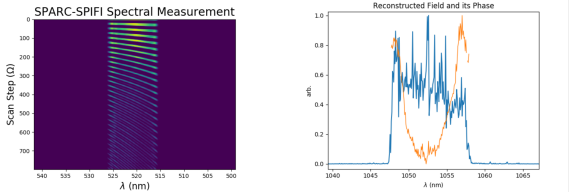
Top Left: Linear imaging MTF for incoherent imaging and SPIFI. Zernike patterns shown for each case (courtesy, R. Bartels, CSU). **Top Right:** Linear first and second order SPIFI images. **Bottom Right:** Nonlinear imaging MTF for 3P-LSM and 3P-SPIFI. Zernike patterns shown for each case (courtesy, R. Bartels, CSU). **Bottom Left:**Two-photon SPIFI image, first and second order of mitotic HeLa cell, reproduced from [1].

Wavelength Domain SPIFI (WD-SPIFI)

Spectral phase and amplitude reconstruction and compensation (SPARC)



SPARC uses a 4F zero dispersion system as shown above, that is placed in-line to the microscope, and ultimately becomes the WD-SPIFI unit. Pulse measurement is performed by scanning a slit at the focal plane of the WD-SPIFI system, and spectrally resolving the frequency-doubled signal at the focal plane of the objective. The spectrally resolved SPARC signal can then be reconstructed via an iterative phase retrieval algorithm. to find the amplitude and phase of the pulse. An example of a pulse measurement is shown for an ANDi fiber laser[2,3]. GDD is compensated by pushing the second grating G2, off of F. Advantage: By using a single-pass grating system, a high transmission efficiency is readily achieved. Greater than 90 percent transmission from off-the-shelf components is now routine. Disadvantage: we are only correcting for GDD, the TOD of the SPARC system adds to the residual TOD of the glass already in the system.

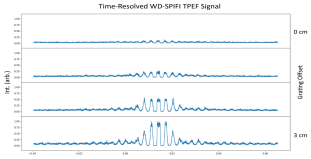


Left: Instead of a scanning slit in SPARC, a SPIFI mask is used. This reticle modulating the wavelength domain is called WD-SPIFI. Spectrally resolving the frequency doubled light at each step of reticle rotation yields a SPARC-SPIFI measurement.
Right: Preliminary results of running the SPARC-SPIFI measurement through Relaxed Averaged Alternating Reflections (RAAR) to reconstruct spectral amplitude and phase shows expected general shape of both amplitude and phase. Refinement to the algorithm due to complexity of solving SPIFI modulation vs. slit modulation is in progress.

WD-SPIFI Pulse optimization in the time domain

Rapidly spinning the SPIFI reticle in WD-SPIFI as is done in SD-SPIFI enables time resolved measurement of the spectral modulation. This has multiple uses, the first of which is live pulse optimization via a nonlinear signal.

- **Right:** Time traces of two-photon excitation fluorescence signal at the specimen plane. The signal provides real-time feedback for GDD compensation through the imaging system.
- Moving the output grating (G2) of the 4F compressor compensates for residual GDD from the ANDi laser, and all subsequent optics.
- The beam is spatially chirped but not significantly (Beam aspect ratio is $\simeq 1$). $\simeq -40k\,fs^2$ of GDD is added for our system (laser plus imaging system).

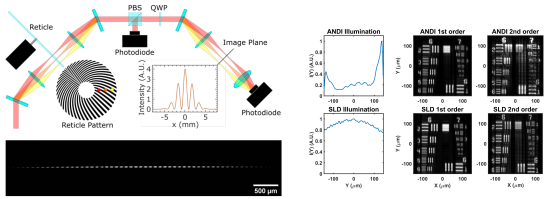


WD-SPIFI and Spatial Domain SPIFI (SD-SPIFI)

WD-SPIFI Linear Imaging

WD-SPIFI spectrally encodes frequency modulation into a line cursor, just as is done in SD-SPIFI.

- Enables linear imaging via free-space propagation or through a fiber, requiring only a grating and lens near the sample plane.
- Requires no system modifications from SPARC-SPIFI nor time domain WD-SPIFI pulse optimization.

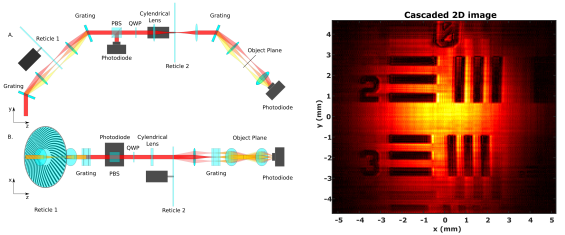


Left: Schematic of WD-SPIFI imaging and modulated line cursor at image plane.
Right: WD-SPIFI images in the 1st and 2nd orders using 2 sources: ANDi fiber laser and Super Luminescent Diode (SLD).

2D WD-SD SPIFI Imaging

Combining WD-SPIFI with SD-SPIFI enables cascaded SPIFI 2D linear imaging, single element detection.

- Spectral frequency modulation occurs within the 4F system in WD-SPIFI.
- Concurrently spatial frequency modulation occurs with a cylindrical lens and another SPIFI reticle in SD-SPIFI.
- Before the sample, a grating is used to disperse the spectral domain perpendicular to the 1D spatial dispersion of the cylindrical lens.
- A 2D light sheet is projected onto the sample. with one dimension encoded with spectral modulation and the other encoded spatially.
- 2D image reconstruction is achieved via 2D FFT



Left: Schematic of WD-SD-SPIFI system in the YZ and XZ planes, demonstrating source extension and modulation in both dimensions.
Right: 2D Image of a USAF test target captured using WD-SD-SPIFI (raw data). Image is 200x200 pixels

References

1. J. Field et. al., PNAS, doi/10.1073/pnas.1602811110 (May 2016)
2. A. Motz et. al., Optics Letters, vol. 44 , No.9, 2085-2088 (2019)
3. A. Motz et. al., Vol. 28, No. 1 / 6 January 2020 / Optics Express, 405-424