Stain-Free Detection and Quantification of Bone Marrow Fibrosis Using Two-Photon Excitation and Second Harmonic Generation Microscopy

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Background
Bone marrow fibrosis is routinely assessed in the diagnostic work-up and prognostic evaluation of patients with known or suspected myeloproliferative neoplasm (MPN). Conventional evaluation of bone marrow fibrosis in cases of MPN is performed on reticulin- and trichrome-stained slides and is semi-quantitative and subjective. MPN grading systems, including the recently revised European consensus (EC) scoring system that uses a 0-3 scale, have been shown to have numerous limitations, including interobserver variability due to the subjective nature of scoring stained slides.

Materials and Methods
Bone marrow core biopsy samples submitted with an indication of MPN were selected for study inclusion. The European consensus scores of the study samples included 0, 2+, and 3+ fibrosis. Unstained 4µm sections of tissue sections underwent 2PE/SHG using the Genesis® 200 (Histoindex Pte. Ltd., Singapore). The Genesis® 200 utilizes an femtosecond erbium fiber laser and a 20x objective to acquire multifocal images (800µm² total area) from each sample, resulting in a spatial resolution of approximately 0.2µm.

Second harmonic generation (SHG) occurs nearly simultaneously with 2PE; two photons interacting with a focal point of the laser where photon density is greatest, resulting in less risk of tissue damage. In addition, 2PE allows for stain-free quantification of unstained tissue structures as a result of intrinsic autofluorescence of collagen fibers (p = 0.008) demonstrated the most significant degree of correlation. Interestingly, FLD using a stereology-based approach, also showed a significant correlation (p = 0.001) with the EC score. Cross-linking of fibers however; several parameters can now be evaluated, as well as compared, to the biology of MPNs.

Conclusions
2PE/SHG imaging imaging, the tissue sections were stained with reticulin and scanned using the Aperio ScanScope (Leica Biosystems, Buffalo Grove, IL). Fiber length density (FLD) was calculated using an approach adapted from stereology. To calculate FLD, the number of reticulin-stained fibers that crossed over a fixed line distance of 239.6µm was manually counted (Figure 4).

Table 1. Correlation between 2PE/SHG image analysis and European consensus score

<table>
<thead>
<tr>
<th>2PE/SHG parameter</th>
<th>FLD</th>
<th>EC score</th>
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<tbody>
<tr>
<td>Total number of fibers</td>
<td>0.054</td>
<td>0.001</td>
</tr>
<tr>
<td>Total length of fibers</td>
<td>0.082</td>
<td>0.001</td>
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<tr>
<td>Aggregated fiber percentage</td>
<td>0.016</td>
<td>0.001</td>
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Figure 4. Fiber length density (FLD) is measured by manually counting the number of fibers that cross a fixed line distance (one arm of a fixed green square). The arrow points to a fiber that would be included in the FLD calculation.

Disclosure Statement
There are no relevant conflicts of interest to disclose.