Sharpness Search Algorithms for Automatic Focusing in the Scanning Electron Microscope

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The scanning electron microscope's transition from a research device to a common industrial tool has increased the need for instrument automation, both in conventional and remote microscopy [2]. Traditionally, autofocusing research has concentrated on finding an optimal sharpness measure, which is then applied over a range of focal lengths and the focal length with the maximum sharpness is chosen as the best focus [3]. The present work investigates the use of more sophisticated sharpness search algorithms which decrease search time without sacrificing the sharpness of the final image.

Four sharpness measures were evaluated based on their robustness to noise, applicability to different specimens, implementation cost, and adherence to the strict unimodality property. A *strictly unimodal* sharpness measure has a single peak at the best focus and is strictly decreasing away from this peak. Strict unimodality is particularly important to the success of more sophisticated search algorithms. The sharpness measures considered were based on the image gradient, sum of specific frequency domain components, image auto-correlation, and image variance.

The *gradient measure* was found to be the most susceptible to noise, while *variance measure* was largely insensitive to noise. The *auto-correlation measure* was usually strictly unimodal but had poor reproducibility, and while the *frequency domain measure* performed well, the implementation cost of performing frequency domain transforms in software was significant. The variance measure was chosen as the primary sharpness measure for this work because of its strict unimodality regardless of noise, as well as its simple implementation. Signal-to-noise (SNR) ratio and objective lens hysteresis effects are important concerns when developing sharpness search algorithms. In this work, SNR issues were addressed by choosing a moderate level of hardware noise reduction and using a robust sharpness measure. Hysteresis was compensated for by resetting the focal length between iterative sweeps and by direct calculation of a hysteresis offset when returning to the best focus following a focus sweep. This hysteresis offset was calculated using a new technique based on the relative sharpness of images obtained during and after the sweep.

The *fixed step-size search* required the most images for a given accuracy. Stopping the search after detecting a sharpness peak improved performance but is applicable only under high SNR conditions. The *iterative search* (using multiple sweeps at decreasing step-sizes) required fewer image captures but additional hysteresis corrections. Following these searches interpolation can be used to further improve the estimated best focus. A new interpolation technique was developed which uses a model of the image variance as a function of defocus and was found to be reasonably successful under most test conditions, but adds significant overhead to the search.

The *variable step-size search* attempts to decrease the step-size during the sweep as the sharpness increases. This technique requires careful initial step-size choice, and is less effective in low SNR conditions. The *Fibonacci search* partitions the search space into segments and iteratively reduces the search space using additional, carefully placed sharpness measurements. This technique requires the fewest images, but like the variable step-size search, it relies heavily on the strict unimodality property.

The search algorithms were tested on various standard and non-biological samples, using both stored focus series and live images on a LEO 440. Their results were verified against maximal sharpness and human assessments of "best focus".

This work has investigated the potential of more sophisticated sharpness search algorithms which require slightly higher SNR requirements but significantly fewer image captures, and introduced novel hysteresis correction and interpolation techniques [1]. The Fibonacci search using a variance sharpness measure is robust and efficient for rapid fine focusing, and combined with the other methods could create a complete full focusing solution.

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References

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