# Digital Composite Imaging for High Resolution Specimen Analysis

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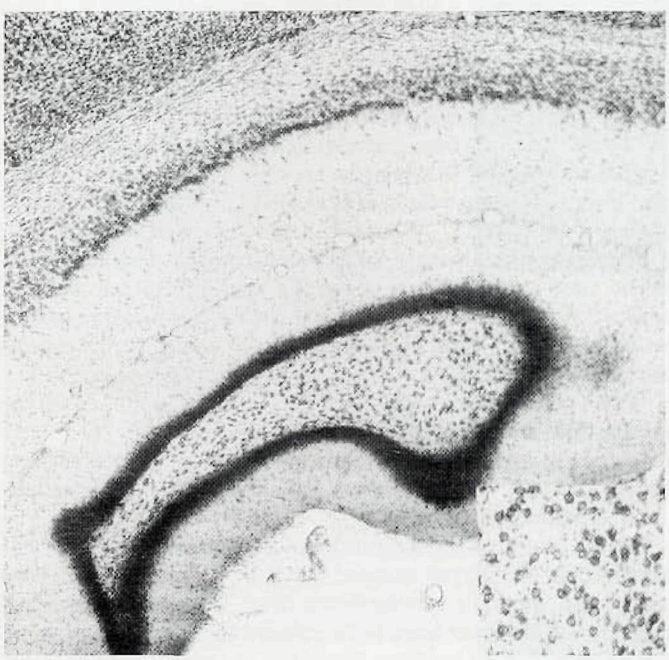
RECENT ADVANCES IN DIGITAL IMAGE PROCESSING hardware providing very high pixel resolutions can be exploited to solve problems in correlating high magnification feature analysis with specimen macrostructure. In neuroscience, for instance, analyses such as neuron counting which are performed at high magnification often need to be correlated with the overall structure of the specimen. Typically these procedures involve tediously moving between very low and very high power objectives. Once at high power, the positional knowledge of the objects of interest can be difficult to maintain.

By integrating high resolution image capture with precise microscope automation, a digital composite image of the specimen can be created. Such composite images show the researcher a high resolution "map" of the specimen, facilitating the analysis of both macro- and microstructure characteristics from a single view.

### Generating the Composite Image

An optical microscope fitted with a motorized stage and focus is coupled to a high resolution image analysis system, capable of driving the motorized stage with sub-micron accuracy. For the example shown in the figure, we used a Leica DM RXE microscope connected to a Quantimet Q600HR high resolution image analysis system. A high power objective is selected, and its magnification is calibrated by the image analysis system. The field of view of the video camera at this magnification is then calculated and used to generate an automated scan of the specimen. The scan pattern consists of a number of fields of view in the X and Y directions of the motorized stage, and the step size between fields is matched to the video field of view. An alignment procedure is used to ensure co-linearity of the stage and image sensor axes.

Once the specimen is positioned, the automated scan is initiated. An automatic focus is performed at each field to achieve precise specimen focus throughout the scan. Successive video fields of view are then digitized and positioned at the appropriate locations in the composite image. When complete, this composite image shows the entire scanned area at the resolution of a single, high-power field of view.



*Composite 2K x 2K pixel image generated by a 4 x 4 field scan using a standard resolution video camera. At the lower right is a detail of a single video field of view in the composite image.*

The high resolution image analysis system then allows both macro- and microstructures to be characterized directly from the composite image, without tedious magnification changes. A dynamic range of feature sizes beyond that of the single video field of view can be measured accurately and automatically. Concerns regarding specimen registration between magnifications are also alleviated, since both macro- and microstructures can be analyzed within the same composite image. Further, the digital map can be used to interactively select an area of interest, which can then be automatically relocated by the image analysis system for further review.

### Summary

Digital composite imaging provides a useful technique for both the visualization and analysis of specimens examined with the microscope. A major benefit is the very large field of view that can be achieved in a single image with the optical properties of a high resolution objective. Excellent flatness of field and illumination spread are also obtained. The wide dynamic range provided enhances image analysis applications in areas, such as neuroscience and the measurement of long fibrous materials, where maximum resolution and very large fields of view are required. ■