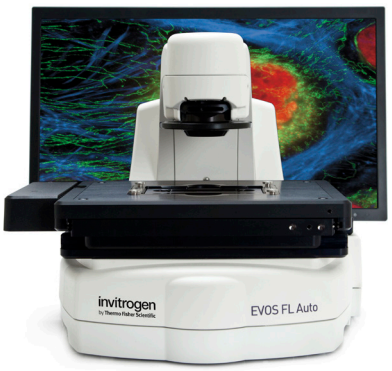
Image tiling and stitching using the EVOS FL Auto Imaging System

Introduction

The Invitrogen™ EVOS™ FL Auto Imaging System is a fully automated, digital, inverted multi-channel fluorescence and transmitted-light imaging system with outstanding workflow efficiency. Designed to meet demanding requirements over a broad range of applications, it supports high-resolution mosaic tiling, multiple-position well scanning, cell counting with thresholding, and time-lapse studies.

The EVOS FL Auto Imaging System allows the acquisition of multiple images at high magnification and the stitching of images together for high-resolution mapping of a large area of sample. This functionality is ideal for analyzing tissue sections or stem cell colonies, or viewing every cell in the well of a 96-well plate. With separate monochrome and color cameras, the EVOS FL Auto Imaging System provides high-resolution capture of both fluorescent and colorimetric images. In this application note, a series of images were captured at 10x magnification of an intestinal section of a transgenic mouse ubiquitously expressing mCherry. Following capture, the images were stitched together to show the entire tissue section.



Materials

- Invitrogen™ Novex™ mCherry Rat Monoclonal Antibody (Cat. No. M11217)
- 4% paraformaldehyde
- Peroxidase Goat Anti-rat IgG Secondary Antibody
- EVOS Light Cube, Texas Red™ (Cat. No. AMEP4655)

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Methods

A transgenic mouse expressing mCherry in all tissues was sacrificed and the intestinal tissue was isolated and fixed in 4% paraformaldehyde. mCherry antibody was used at 1:15,000 followed by detection with the an anti-rat IG secondary antibody conjugated with peroxidase. Tissue sections were incubated in the peroxidase substrate solution until the desired stain intensity developed. Images were collected and stitched with the Scan feature on the EVOS FL Auto Imaging System using either the color or monochrome camera.

Results and Discussion

The EVOS FL Auto Imaging System can take individual images over a specified area of interest (Figure 1), then seamlessly stitch the images together to create a single image of the entire section or area of scan (Figure 2). The optical resolution and image quality of each panel is maintained in the full image (Figure 3).

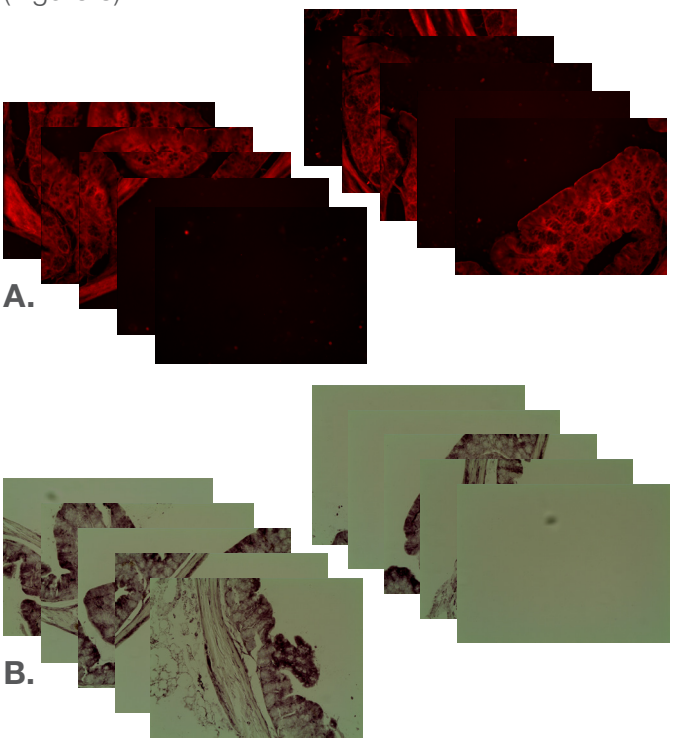

Figure 1. A selection of 10 of the 63 images collected for the image scan. mCherry expression was detected through fluorescence **(A)** or colorimetric **(B)** peroxidase staining. Images were collected at 10x magnification.

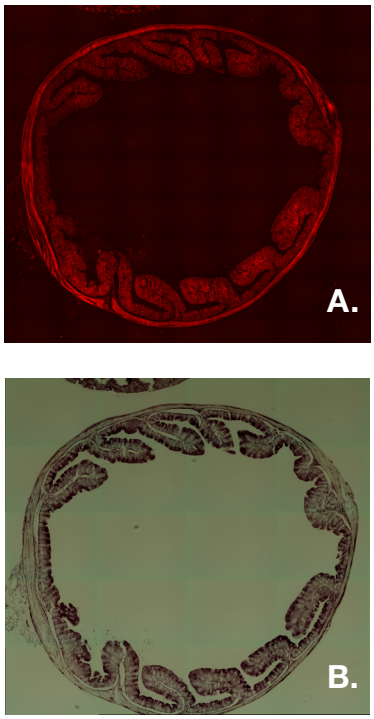

Figure 2. A series of 63 images taken at 10x magnification stitched together to create a single merged image. mCherry expression was detected through fluorescence **(A)** or colorimetric **(B)** peroxidase staining.

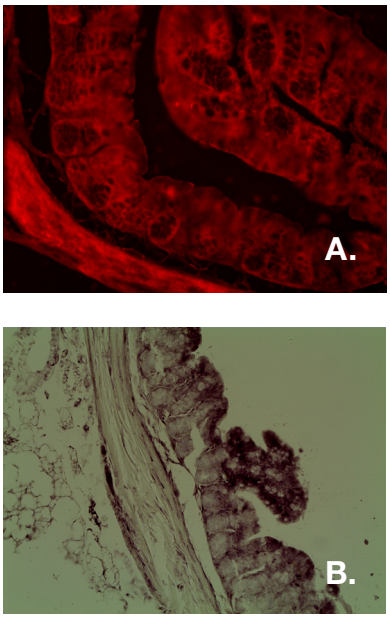

Figure 3. Individual image of the stitched image taken at 10x magnification to show resolution of the image is maintained. mCherry expression was detected through fluorescence **(A)** or colorimetric **(B)** peroxidase staining.

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