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Three-Dimensional Virtual Microscopy of Colorectal Biopsies

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• Conventional optical microscopy of specimens from colorectal biopsies commonly produces diagnostic errors due to incomplete sampling or poor orientation. Obtaining additional sections or re-embedding may help avoid these errors, but can prolong turnaround time. We describe new technology, which incorporates exhaustive sectioning, 3-dimensional reconstruction, and virtual microscopy, that may eliminate these problems by enabling pathologists to rapidly examine entire specimens and convert poorly oriented mucosa to well-oriented mucosa.

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Conventional optical microscopy of specimens from colorectal biopsies examines only a few histologic sections per day. This widely used method of examination is suboptimal for many reasons. Diagnostic errors due to incomplete sampling are common because only a small fraction of the specimen is visualized.[1–5] Diagnostic errors due to poor orientation may also occur because many institutions randomly embed these specimens.[6] Furthermore, attempts to avoid these errors by obtaining additional sections or by re-embedding can prolong turnaround time by 1 or more days. We describe new technology, which incorporates exhaustive sectioning, 3-dimensional reconstruction, and virtual microscopy, that may eliminate these problems by enabling pathologists to rapidly examine entire specimens and convert poorly oriented mucosa to well-oriented mucosa.

MATERIALS AND METHODS

On day 1, a 76-year-old man underwent surveillant colonoscopy for polyps. A putative diminutive polyp was noted in the cecum. Multiple biopsies of the putative diminutive polyp were performed using cold jumbo forceps, and all material was transferred to a single container without placement on a solid medium. On day 2, routine histologic processing and conventional optical microscopy were performed. Orientation was attempted at paraffin-embedding. The paraffin-embedded specimen was serially sectioned until the full cross-sectional area of the specimen was believed to be exposed. These sections were discarded. Six sections were then cut at intervals of 3 μm and retained for conventional optical microscopy. Final stages of processing were performed with the Tissue-Tek DRS 2000 Automated Slide Stainer and Tissue-Tek SCA Coverslipper (Sakura, Torrance, Calif) and an electronic typewriter to label the slides. These sections showed normal mucosa with 1 small, poorly oriented focus containing obliquely sectioned crypts (as read by M.L.W., data not shown).

Additional serial sections were requested. At the patient's institution, like in many academic institutions, routine requests for additional serial sections are completed the following day. On day 3, additional serial sections to be used both for further conventional optical microscopy and for virtual microscopy were obtained. The specimen was exhaustively sectioned at intervals of 3 μm, and these sections were retained, placed on electronically labeled slides, stained, and coverslipped with the same instruments from Sakura, scanned and digitized using the HI-Scope system (3DHISTECH, Budapest, Hungary), then analyzed using the 3D-Scope program (3DHISTECH). Complete technical details of the recently developed HI-Scope system and 3D-Scope program are described elsewhere.[7] Briefly, the HI-Scope system is a fully automated digitizer capable of rapidly digitizing hundreds of slides at a time, at high resolution (Figure 1, A and B). The data are stored on an IBM TotalStorage server system (IBM, White Plains, NY), including an IBM X series 225, a double Xeon processor server, and Fast 200 redundant arrays of inexpensive disks (700 GB). The 3D-Scope program evaluates the 2-dimensional digitized images of serial sections with virtual 2-dimensional and 3-dimensional microscopy. The complete array of 2-dimensional digitized images of serial sections may be displayed simultaneously, providing a magnifiable gallery of essentially the entire specimen. The 2-dimensional images may be automatically stacked and aligned to form a magnifiable 3-dimensional image of the specimen. Users may interact with the program to rotate the 3-dimensional image about any axis and to simulate serial sectioning of the 3-dimensional image along any plane. The virtual microscopy is performed on an IBM Medical Workstation computer and displayed on a T221 high-resolution monitor (22-inch diagonal screen, 4000 × 2500 pixels, 200 dots per inch). The HI-Scope system and 3D-Scope program together currently cost approximately US $120 000.

RESULTS

From the gallery of 2-dimensional images (Figure 2, A), a tubular adenoma in well-oriented mucosa, diagnosed according to standard criteria,[8] and the poorly oriented focus were immediately evident (Figure 2, B) in the image

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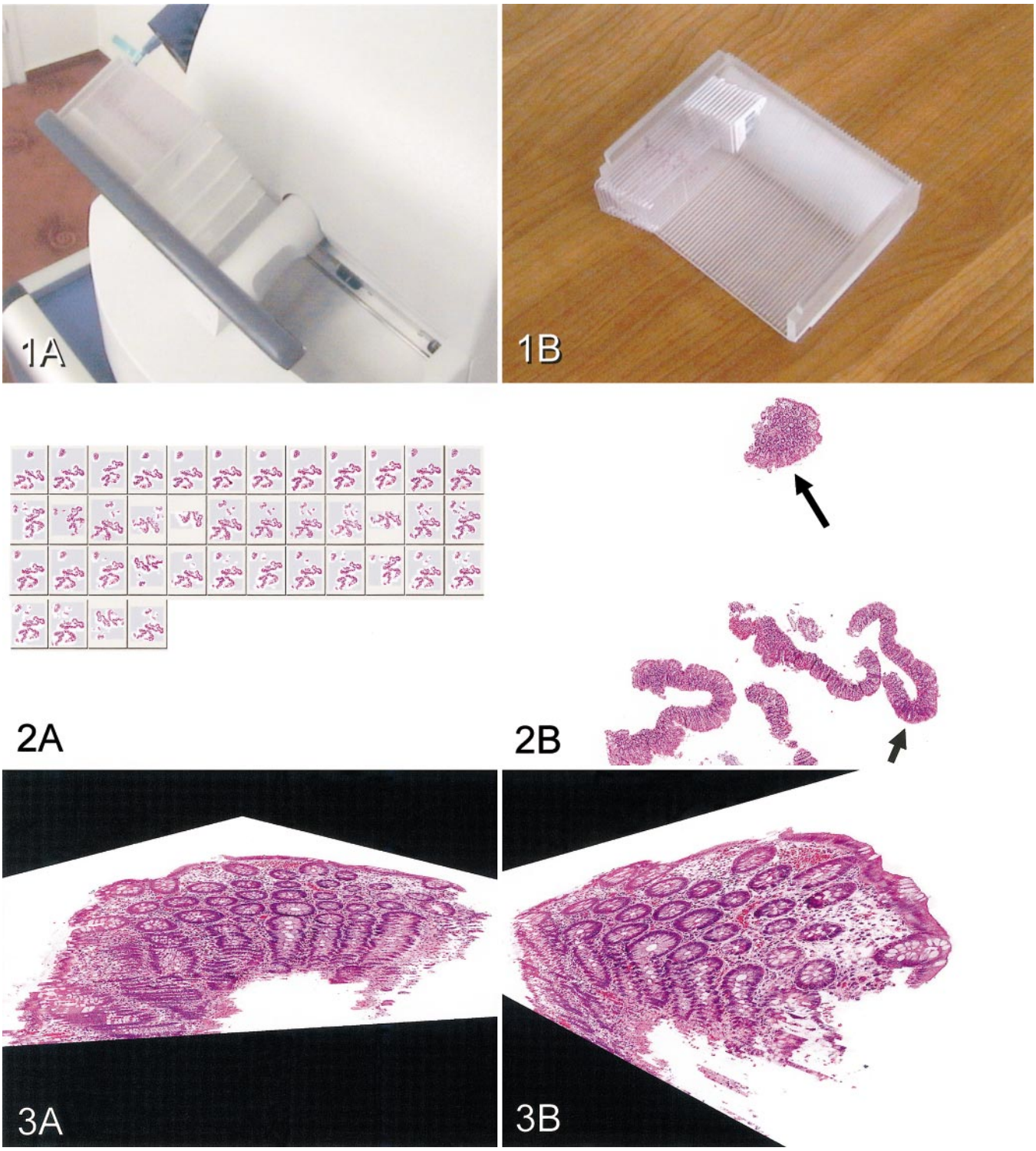

Figure 1. *HI-Scope system. A, Automated loading and unloading of slides. B, Removable slide holder.*

Figure 2. *Gallery of exhaustive sections of specimen from cecal biopsies. A, Panoramic view shows essentially the entire specimen (hematoxylin-eosin, original magnification ×20). B, View at intermediate magnification shows adenoma (short arrow) and poorly oriented focus (long arrow) (hematoxylin-eosin, original magnification ×100).*

Figure 3. *Three-dimensional virtual microscopy. A, Virtual sectioning along proper plane simulates well-oriented mucosa with longitudinally sectioned crypts (hematoxylin-eosin, original magnification ×200). B, Virtual rotation about a selected axis provides a different perspective (hematoxylin-eosin, original magnification ×200).*

Table 1. Comparison of Conventional and New Techniques: Time		
	Conventional Technique	**New Technique**
Sectioning, 3 sections/slide	6 sections/1 min	40 sections/10 min
Labeling	2 slides/2 min	15 slides/2 min
Automatic staining	2 slides/30 min	15 slides/30 min
Automatic coverslipping	2 coverslips/1 min	15 coverslips/1 min
Scanning and digitizing	. . .	120 min
Interpreting	2 slides/1 min	Gallery of images/1 min
Repeating as necessary	120 min	. . .
Three-dimensional reconstruction	. . .	30 min
Reorienting	120 min	1 min
Total	**275 min**	**195 min**

Table 2. Comparison of Conventional and New Techniques: Direct Cost*		
	Conventional Technique†	**New Technique**
Sectioning ($0.24/min technologist's time)	$0.24 × 2	$2.40
Slides ($0.22/slide)	$0.44 × 2	$3.30
Coverslips ($0.16/coverslip)	$0.32 × 2	$2.40
Interpreting ($1.51/min pathologist's time)	$1.51 × 2	$1.51
Total	**$2.51 × 2 = $5.02**	**$9.61**

* Costs calculated in US dollars.
† Assumes polyp revealed in first set of additional sections.

of each additional serial section. The possibility of a second lesion coexisting with the adenoma was excluded, even in the poorly oriented focus. Conventional optical microscopy confirmed the presence of the adenoma and poorly oriented focus in all additional serial sections (as read by M.L.W.).

We selectively cropped the poorly oriented focus from the image of 1 serial section, and the corresponding focus from the images of all other serial sections was then automatically cropped. These cropped images were then automatically stacked and aligned to reconstruct a 3-dimensional image of the poorly oriented focus. We interacted with the program to rotate the 3-dimensional image about various axes and to section the 3-dimensional image along various planes until several crypts were sectioned longitudinally (Figure 3). This interaction converted the poorly oriented mucosa to neo-well-oriented mucosa and required only a few seconds. Sections from the neo-well-oriented mucosa appeared normal. All authors viewed all of the images, and all have special interest in gastrointestinal pathology.

For the initial conventional optical microscopy, the cumulative time required for sectioning, staining, coverslipping, labeling, and interpreting was approximately 34 minutes; the corresponding direct cost[2] was US $2.51, although these sections failed to reveal the adenoma and had a poorly oriented focus. For the entire conventional optical microscopy, the corresponding direct cost to detect the adenoma, including additional sections, was US $5.02. Although requests for routine additional sections and for reorienting are commonly completed the next day, these requests could be completed in a few hours or less under ideal circumstances. Assuming that the entire process of obtaining a small set of additional sections could be completed in 2 hours and that successful reorienting could be completed in 2 hours, conventional optical microscopy would have required approximately 275 minutes to obtain and interpret initial sections, routine additional sections, and reoriented sections. For the new technology, the cumulative time required for exhaustive sectioning, staining, coverslipping, electronic labeling, scanning, digitizing, 3-dimensional reconstructing, and interpreting was approximately 195 minutes; the corresponding direct cost was US $9.61 (Tables 1 and 2).

COMMENT

Conventional optical microscopy of specimens from colorectal biopsies examines only a few histologic sections and hence a very small portion of the specimens. Diagnostic errors due to incomplete sampling are common. Such errors are particularly common in the setting of diminutive polyps and inflammatory bowel disease.[1–5] Additional sections of specimens from biopsies of putative diminutive polyps, for which initial sections lack polyps, may contain polyps in more than 30% of cases.[1] Obtaining additional sections may increase the yield of finding granulomas associated with Crohn disease by 50%.[4,5] These data are all based on partial sectioning, rather than exhaustive sectioning, and likely underestimate the utility of additional sections.

Conventional optical microscopy cannot easily convert poorly oriented specimens to well-oriented specimens. Poorly oriented specimens can be re-embedded at different angles to create well-oriented specimens, although in our experience success is achieved somewhat by trial and error. Proper orientation greatly improves diagnostic accuracy.[6] For example, the presence or absence of maturation, which helps differentiate regeneration from dysplasia, is easily determined in well-oriented mucosa but is difficult to determine in poorly oriented mucosa. Features of inflammatory bowel disease that occur deep in the mucosa, such as branched crypts, metaplastic Paneth cells, and basal lymphoplasmacytosis, are best demonstrated with proper orientation. Proper orientation requires the directed efforts and collaboration of endoscopists, their nurses and assistants, pathologists, and histotechnologists[6] and unfortunately may be difficult to achieve in busy and understaffed endoscopy suites or histology laboratories.

The fast rate of solidification of paraffin permits only 4 pieces of mucosa per block to be properly oriented (Mary P. Bronner, MD, unpublished data, April 2004). Endoscopists often submit more than 4 pieces of mucosa per specimen, and these specimens are usually processed in 1 block. Therefore, many institutions routinely produce sections with poorly oriented mucosa.

Furthermore, if findings on the first set of additional sections are noncontributory or if the first trial of re-embedding fails to produce well-oriented mucosa, additional sectioning or re-embedding may have to be repeated. Each procurement of additional sections, with or without re-embedding, may significantly increase turnaround time, depending on the overall workload of the pathologists and histotechnologists. Therefore, conventional optical microscopy may hide critical diagnostic and clinically relevant information regarding polyps and inflammatory bowel disease, and is inefficient if additional sections are obtained or if re-embedding is needed.

We describe new technology, which incorporates exhaustive sectioning, 3-dimensional reconstruction, and virtual microscopy, that may eliminate these problems by enabling pathologists to rapidly examine entire specimens and convert poorly oriented mucosa to neo-well-oriented mucosa. The gallery of 2-dimensional images enabled us to instantly evaluate essentially the entire specimen, nearly eliminating errors due to incomplete sampling and eliminating the need to obtain additional sections. The new technology required approximately 195 minutes to detect the adenoma and convert the poorly oriented focus to a neo-well-oriented focus. Conventional optical microscopy under ideal conditions would have required approximately 275 minutes, assuming that a small set of additional sections could be obtained in 2 hours and that successful reorientation could be achieved in 2 hours (Table 1). Labeling, staining, coverslipping, and even interpreting required the same amount of time between methods, because labeling, staining, and coverslipping were performed as a batch; because the gallery of images provided simultaneous viewing of all sections; and because the polyp was easily diagnosed once displayed in the gallery. The direct costs of the new technology, namely, technologist's time, slides, coverslips, and pathologist's time, were more than that of conventional optical microscopy (Table 2). However, we feel that this cost is justified because the new technology could potentially detect large numbers of polyps that conventional optical microscopy might overlook, and because of the importance of polyps.[1–3]

Recent data indicate that diagnoses rendered by 2-dimensional virtual microscopy correlate well with diagnoses rendered by conventional optical microscopy,[9] and in our study the diagnosis of adenoma was easily rendered by virtual microscopy.

The poorly oriented focus was rapidly and easily converted to a neo-well-oriented focus because virtual microscopy provided immediate feedback. Results of rotation about any chosen axis and of sectioning along any chosen plane were immediately visualized, and the axis or plane could be rapidly and finely adjusted accordingly. Furthermore, we could reconstruct the image and start over as many times as needed. In contrast, conventional reorienting by re-embedding would have sectioned a coarsely and somewhat randomly rotated specimen, with delayed feedback of results. If the initial attempt to reorient failed, subsequent attempts might produce sections that would be difficult to interpret, because each attempt would partially destroy the specimen.

Improvements to the technology are being investigated. The resolution is currently limited by the finite number of sections available and by the fundamentally 2-dimensional nature of digitizing. Exhaustive sectioning at 1-μm intervals would triple the number of sections and 2-dimensional images, and thereby triple the resolution of the 3-dimensional image. With no tissue remaining, only destained sections can be used for cytochemistry or immunohistochemistry, which might impair the final evaluation. Alternatively, a few unstained sections from periodic intervals could be routinely withheld and saved on conventional or charged slides, and ancillary studies could be performed on these sections if necessary without significantly compromising the resolution of virtual microscopy. These precautionary, withheld, unstained sections would help accurately diagnose unexpected lesions, such as lymphoma, melanoma, or neuroendocrine tumor. Currently, the technology cannot retouch defects that are innate to sections with poor quality, such as folds, chatter, variable staining, and contamination.

Approximately 30% of specimens from biopsies of putative diminutive polyps have normal initial sections (M.L.W., unpublished data, November 2004). If incorporated into clinical practice, we propose that the technology be used with specimens that fall into this category. Although technologists would spend more time per specimen, pathologists would not, because after the initial conventional optical microscopy, pathologists would just pull up the images on the computer and render a diagnosis without further using the optical microscope. We are investigating the possibility of automated sectioning, which would automate the whole process without significantly increasing cost.

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