High resolution colonoscopy in live mice

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Endoscopy in humans is a powerful method for physicians to examine the gut for inflammatory or neoplastic changes. In medical and immunological research, animal models of intestinal diseases are established key tools to investigate the mucosal immune system, colitis and cancer development in the gut. Moreover, such models represent valid systems for testing of novel drugs. In the past, mice had to be killed in order to analyze colitis activity and tumor development. The following protocol describes a method to perform high resolution endoscopic monitoring of live mice. Mice developing colitis or colonic tumors are anesthetized and examined with a miniendoscope. The endoscope is introduced via the anus and the colon is carefully insufflated with an air pump. Endoscopic pictures obtained are of high quality and allow the monitoring and grading of tumors and inflammation. In addition, colonic biopsies can be taken. This protocol can be completed within 1 h.

INTRODUCTION

Numerous mouse models of inflammatory bowel disease and colon cancer have been developed in the past (for reviews, see refs. 1–4). These models not only have substantially helped in gaining insights into the pathogenesis of these diseases, but also have been used as model systems of mucosal immune responses by investigating the interplay of different immune cells. In humans, recurrent endoscopic examination of the colon is of utmost importance for the diagnosis and staging of inflammatory bowel diseases and colon cancer. Unfortunately, this method has not been available for routine use in mice, due to the small diameter of the mouse colon, although several attempts have been made to visualize the mouse colon and to assess colon pathology *in vivo*[5–9]. Thus, in the past, it was impossible to examine the gut directly in mice, and the animals had to be killed in order to visualize the colonic mucosa. As a consequence, only indirect parameters such as the weight loss of the animal, food and water uptake or the presence of blood in the feces could be used to obtain some information on colonic inflammation in live mice. However, weight loss in mice can also occur in response to stress or as a result of unrelated infections, especially when using immunocompromised mice.

Here, we provide a protocol for high resolution endoscopy in mice. This protocol yields publication-quality pictures and movies of the colon of mice in terms of health and disease[10,11]. Endoscopic pictures allow the monitoring and grading of the disease. Scoring of colitis activity as well as of tumor size and growth is also possible. A working channel allows the taking of biopsies and the local injection of solutions. Surface staining with methylene blue can enable visualization of the surface of the crypts and the pit pattern architecture of the colonic mucosa. Crypt pattern analysis using dye-aided endoscopy permits discrimination between inflammatory and neoplastic changes. Biopsies yield sufficient tissue for molecular and histopathological analyses. In sum, endoscopy in mice allows the development of colitis and colon cancer to be monitored at high resolution. Manipulations such as local injection of reagents or the taking of biopsies can easily be performed, giving the investigator powerful tools to assess and manipulate disease progression in small laboratory animals *in vivo*. Finally, to improve animal welfare, endoscopy can help to significantly reduce animal numbers needed to gain significant results. Standard endoscopy can easily be performed by a trained graduate student. The taking of biopsies requires help from a colleague. In summary, endoscopy in mice is a feasible and highly useful method to investigate colon pathology.

MATERIALS

REAGENTS

- Specific treated or untreated pathogen free mice of the desired strain
  ! CAUTION All animal experiments have to be performed in accordance with national regulations. Mice should be no younger than 6 weeks to enable introduction of the endoscope without harming the animal.
- Methylene blue (Neopharma, Aschau, Germany) (see REAGENT SETUP)
- Eye ointment Bebanthen (Roche, Basel, Switzerland)
- Sterile isotonic saline (PAA, Pasching, Austria)
- Ketamine (Ketavest 100 mg ml^{-1}, Pfizer, Karlsruhe, Germany)
- Xylazine (Rompun 2%, Bayer Healthcare, Leverkusen, Germany)

EQUIPMENT

- Coloview miniendoscopic system (Karl Storz, Tuttlingen, Germany) (see EQUIPMENT SETUP)
- Endovision Tricam (Karl Storz) (see EQUIPMENT SETUP)
- Monitor (see EQUIPMENT SETUP; Sony, Cologne, Germany)
- XENON 175 (Karl Storz)
- HOPKINS Straight Forward Telescope 0 ° (Karl Storz; see EQUIPMENT SETUP)
- Endoscopic sheath (Karl Storz; see EQUIPMENT SETUP)
- Sony DSR-20MDP (Sony) (see EQUIPMENT SETUP)
- ScenalizerLIVE (can be downloaded online at http://www.scenalizer.com) (see EQUIPMENT SETUP)

REAGENT SETUP

Methylene blue should be diluted with PBS to a final concentration of 1%.

Anesthetic Use a mixture of ketamine and xylazine. Mix 0.6 ml ketamine (100 mg ml^{-1}), 0.4 ml xylazine (20 mg ml^{-1}) and 4 ml saline and inject a volume of approximately 8 µl per gram body weight. **▲ CRITICAL** The solution must be prepared on the day of the experiment. The dose described here is sufficient to keep the mouse anesthetized for at least 30 min.

EQUIPMENT SETUP

Total setup **Figure 1** shows a typical setup (Coloview miniendoscopic system) used for mouse endoscopy.

Camera An endoscopic camera is needed to record endoscopies. Triple chip cameras (e.g., Endovision Tricam) give superior image quality over single chip

cameras. Using a digital camera will make video transfer to the computer easier and prevents loss of data.

Monitor A standard color monitor with a screen size of 30–40 cm is sufficient. The monitor should have at least composite video input and S-VHS input options.

Light An endoscopic cold light fountain (e.g., XENON 175) with adjustable light intensity should be used. Using a light source with an integrated antifog pump allows inflation of the colon without the need for a special device. The air flow can easily be regulated with the standard valve present at the Luer lock adapter of the endoscopic sheath.

Optics An endoscopic straight forward telescope should be used. The fiberoptic light transmission has to be included in the optics. The overall diameter should not exceed 2 mm for mice (e.g., HOPKINS Straight Forward Telescope 0°).

Endoscopic sheath Two different sheaths for the telescope are recommended: one with a small diameter of not more than 3 mm for endoscopy without manipulations and the other with an additional working channel not exceeding 3.5 mm.

Tape recorder A digital tape recorder is needed to store the video files. Professional tape recorders (e.g., Sony DSR-20MDP) give many options, but a consumer digital video (DV) camcorder with a DV-IN port is also fine. Keep in mind that a DV-based recording system has important advantages over MPEG-based DVD systems. DV uses a frame-based compression algorithm generating 25 full pictures per second. MPEG (DVD) uses vector-based compression, meaning that fewer good pictures can be recovered per second.

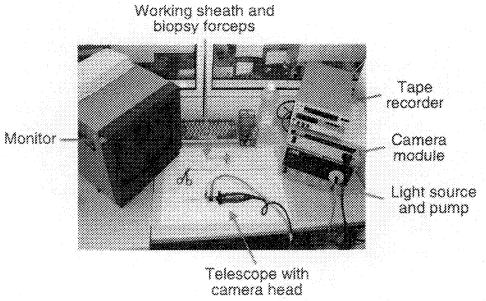

Figure 1 | Experimental setup of the Coloview miniendoscopic system showing endoscopic tools used for mouse examinations.

A computer with firewire-adapter to transfer video material from the tape to the computer. Keep in mind that 1 h of DV-compressed video will need approximately 13 GB of hard disk space. Use software (e.g., ScenalizerLIVE) to grasp individual pictures.

A heating pad to keep mice warm during recovery from anesthesia.

A scale to determine the correct weight of the animals to calculate the anesthetic dose necessary.

PROCEDURE

Preparing the endoscopic setup ● TIMING Approximately 5 min

1| Before anesthetizing the animals, set up the endoscopic device. Sterilize the endoscope, clean the telescope lens with water and ethanol and set the focus so that objects at a distance of 3–5 mm give a crisp picture. Using a thin coating of lubricant on the endoscopic sheath will facilitate inserting the scope.

2| Attach the Luer lock of the endoscopic sheath to the air pump and adjust the valve of the Luer lock adapter to create a slow constant flow of air.

▲ CRITICAL STEP Check function of the air pump by putting the scope into water. One small bubble per second is ideal. Setting the air flow too high can harm the animal by severely inflating the gastrointestinal tract.

Anesthetizing the animal

3| Inject a mixture of ketamine and xylazine i.p. Use the lowest possible dose necessary for sedation to perform the endoscopy. This will guarantee a minimal influence of the procedure on your experimental outcomes.

▲ CRITICAL STEP Be sure that the mice are fully anesthetized before introducing the endoscope. If mice start moving during endoscopy, the device may cause injuries to the colonic mucosa. Check by carefully pulling on one leg. If the mouse does not struggle, it is completely anesthetized. Apply a small amount of gel to the eyes of the mouse to protect the corneas.

Endoscopy

4| You can now perform standard endoscopy to evaluate colon pathology (option A), take biopsies from live mice (option B) and/or stain the mucosa with methylene blue (option C).

(A) Standard endoscopy for evaluating colon pathology

(i) Take an anesthetized mouse and put it on a clean paper with its ventral side up. Hold the fur just above the anus of the mouse with one hand and the scope in the other hand.

(ii) Carefully insert the endoscope into the rectum. The air flow of the pump should be slow while keeping the colon inflated. One small bubble in water per second is usually ideal.

(iii) Carefully push the endoscope as far as possible under visual control. The endoscope can usually be inserted up to 4 cm. At that point, the colon in mice makes a curve (flexure) which cannot be passed with a rigid scope.

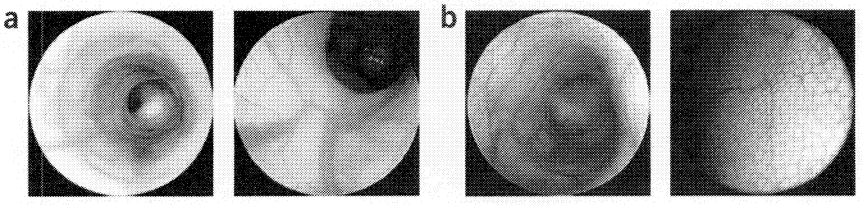

Figure 2 | *In vivo* high resolution endoscopy and chromoendoscopy of a healthy mouse. Representative endoscopic pictures showing the colon of a healthy mouse. (**a**) Note the smooth and transparent mucosa, the normal vascular pattern and the regular crypt pattern in the methylene blue stained colon. (**b**) For chromoendoscopy (lower two pictures), the colon mucosa was stained with methylene blue to visualize the crypt pattern.

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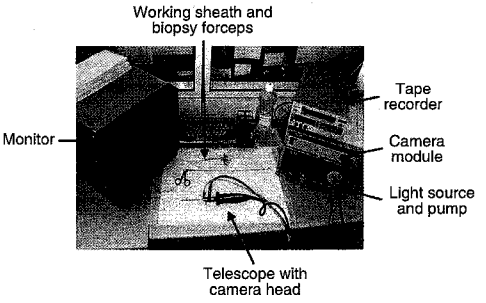

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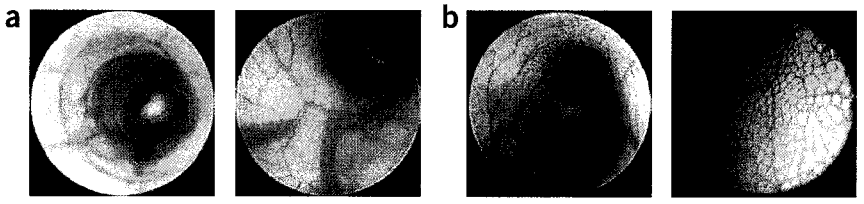

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(ii) Carefully insert the endoscope into the rectum. The air flow of the pump should be slow while keeping the colon inflated. One small bubble in water per second is usually ideal.

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TABLE 1 | Endoscopic colitis score

	Murine endoscopic index of colitis severity (MEICS)				
	0	1	2	3	Total
Thickening of the colon	Transparent	Moderate	Marked	Non-transparent	0–3
Changes of the vascular pattern	Normal	Moderate	Marked	Bleeding	0–3
Fibrin visible	None	Little	Marked	Extreme	0–3
Granularity of the mucosal surface	None	Moderate	Marked	Extreme	0–3
Stool consistency	Normal + solid	Still shaped	Unshaped	Spread	0–3
					Overall: 0–15

Endoscopic colitis score based on the observed signs of inflammation. The MEICS consisted of five parameters, as indicated.

(iv) If endoscopies should be recorded, start recording as you withdraw the endoscope slowly. While recording in DV, the camera picture, which is a standard PAL (phase alternating line) signal, will give rise to 25 single software compressed pictures per second (approximately 30 when using the National Television Systems Committee (NTSC) format), thereby creating a large pool of pictures from which one may select the best images later on (see **Fig. 2** for representative pictures of a healthy mouse and **Fig. 3** and **Fig. 4** for pictures of colitis and colon tumors, respectively). Upon reaching the anus, stop the recording.

(v) If the mice should be scored for colitis or tumor development during the endoscopy, note differences in the thickness of the bowel wall, changes in blood vessel integrity, mucosal surface, stool consistency or the presence of fibrin, as shown in **Table 1**.

(vi) Finally, remove the endoscope and put the mouse back into the cage.

(B) Taking colonic biopsies from live mice

(i) Exchange the sheath of the endoscope to allow the use of a biopsy forceps. Insert the biopsy forceps until the forceps is just inside the examination sheath. Adjust air flow as described above (Step 2).

(ii) Insert the scope as described in Step 4A and move forward to the point of interest.

(iii) While holding the mouse in one hand and the scope in the other hand, get a colleague's help to push forward the biopsy forceps and grasp the tissue (see picture series in **Fig. 5**).

▲ CRITICAL STEP The colon wall of a healthy mouse is very thin. To avoid perforation of the colon, take extreme care to grasp the tissue at a flat angle. Perforation is usually not a problem when using mice with colitis or when grasping tumor tissue, as the colon wall of these mice is much thicker.

(iv) Open the biopsy forceps and with a needle take the tissue piece for further use. If the tissue should be stored, put it into a cryovial and freeze it in liquid nitrogen. Even the smallest available biopsy forceps will allow you to obtain tissue pieces that are sufficient for histochemical analysis and quantitative PCR assays.

(v) If further biopsies from the same mouse are needed, grasp the tissue that is located proximal to the site of the biopsy first in order to avoid impacting the view, as some bleeding may occur after previous biopsies.

(C) Staining of the mucosa with methylene blue

(i) Carefully insert the endoscope into the rectum and push it forward to the flexure as described in Step 4A.

(ii) Remove the air tube and inject 0.5 ml of a 1% solution of methylene blue with a syringe slowly into the colon while moving the endoscope backwards.

(iii) Remove the endoscope and wait for 3 min. Flush the examination sheath several times with water to remove residual methylene blue from the endoscope.

(iv) Attach the air pump again and perform the endoscopy as described in Step 4A. Note the crypt pattern, mucosal erosions, ulcers and aberrant crypts.

Finishing the endoscopic examination

5| After the endoscopic examination, mice should be put on a heating pad to keep them warm during recovery from anesthesia. Anesthetized mice should be allowed to wake up in a separate enclosure to protect them from nonanesthetized cagemates.

Data analysis

6| Colitis scoring. Any colitis can be reliably scored by anticipating differences in the colonic mucosa, as shown in **Figure 3**. We developed a colitis score, denoted by MEICS (murine endoscopic index of colitis severity). The MEICS system consisted of the following five parameters: thickening of

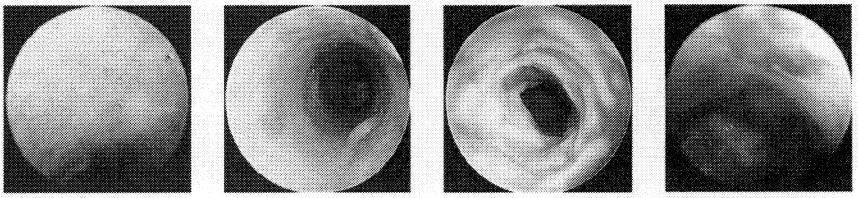

Figure 3 | Representative endoscopic pictures of a mouse with colitis showing signs of severe inflammation. Note bleeding mucosa, altered vascular pattern, intransparent mucosa, abundant fibrin and loose stool in these pictures.

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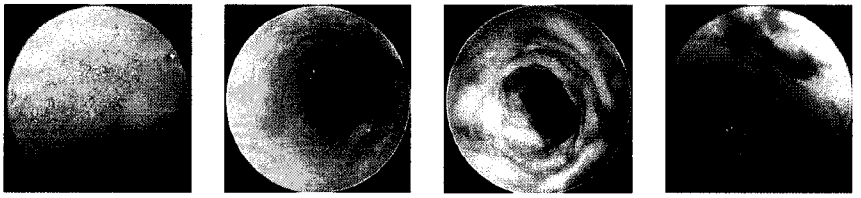

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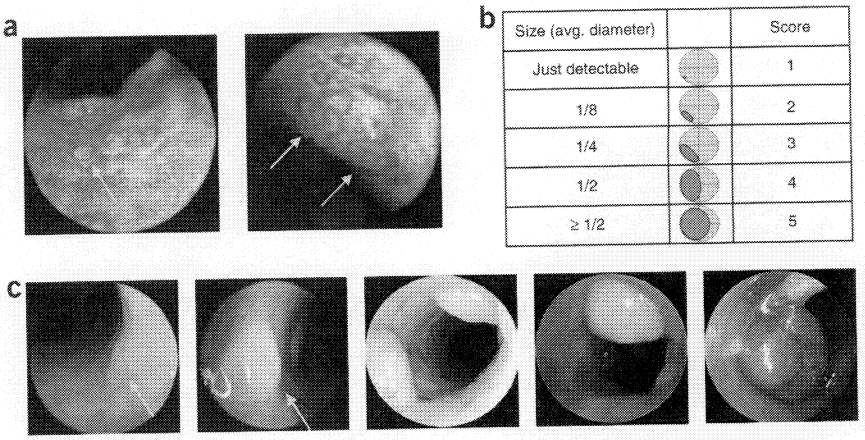

Figure 4 | Endoscopic scoring of tumor development in mice. (**a**) Methylene blue staining of the colonic mucosa during endoscopy of mice developing tumors. Shown are representative pictures of chromoendoscopic signs of early neoplasias (ACFs) indicated with arrows. (**b**) The grading of tumor size relative to the circumference of the colon. (**c**) Representative endoscopic pictures showing the development of colon tumors during the course of the experiment. Shown are tumors graded as sizes 1–5.

7| Tumor scoring. Tumor development can be scored by the total number of tumors or the size of tumors, as shown in **Figure 4**. Differences in tumor numbers are indicative of differences in tumor induction, whereas differences in average tumor sizes suggest differences in tumor progression. Tumors observed during endoscopy should be counted to obtain the overall number of tumors. Tumor sizes should be graded as follows: Grade 1 (very small but detectable tumor), Grade 2 (tumor covering up to one-eighth of colonic circumference), Grade 3 (tumor covering up to one-fourth of the colonic circumference), Grade 4 (tumor covering up to half of the colonic circumference) and Grade 5 (tumor covering more than half of the colonic circumference). An account of tumor scoring *in vivo* in mice using this technique has recently been published[10,14].

? TROUBLESHOOTING

Problems during endoscopy usually decrease with the experience of the investigator. Some of those problems are as follows:

Mortality of animals

Mortality of animals during the procedure can have several causes. First, it can be due to a perforation of the colon during routine endoscopy or upon taking of biopsies. Be careful not to push the endoscope with too much force if you feel resistance. Moreover, when taking biopsies, care should be taken to hold the biopsy forceps at a flat angle. Second, mortality can be due to the bad health status of the animals. Moribund animals with severe wasting are very sensitive to anesthesia and sometimes do not recover after endoscopy. Third, severe inflation of the gut during the procedure can harm the animals. If you observe marked inflation, immediately decrease the air flow of the pump.

Bad visibility

Bad visibility can be due to feces or insufficient air flow to inflate the colon. If feces is a problem, flush a small volume (100 µl) of saline into the colon using a Pasteur pipette. If feces or blood is coating the optical lens, withdraw the endoscope, clean it with water and reintroduce it.

ANTICIPATED RESULTS

We have developed a novel endoscopic procedure allowing high resolution imaging of the colon in live mice (**Fig. 2**). This method allows fascinating new insights into the pathogenesis of murine colitis and colon cancer by monitoring and scoring of colitis activity and colon cancer (**Figs. 3**, **4**). Furthermore, mouse colonoscopy permits colonic biopsies to be obtained from single tumors (**Fig. 5**). Since the investigator may analyze the success of the experiments early on and is able to monitor disease activity and extent *in vivo*, this approach may also help to limit the costs and the number of mice per experiment.

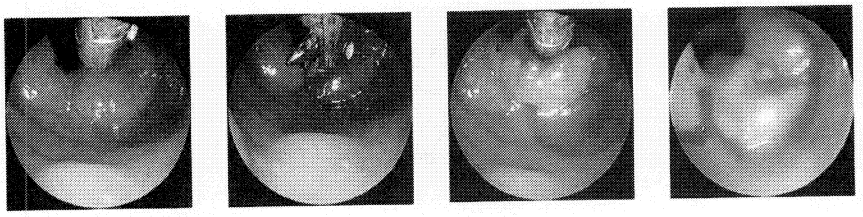

Figure 5 | Getting biopsies from the colon of a live mouse. Representative pictures taken during bioptic sampling of a tumor during routine endoscopic monitoring of a mouse. Tumor biopsies were snap frozen in liquid nitrogen and cut for staining.

Murine experimental models provide important tools to investigate the pathogenesis and therapies of chronic

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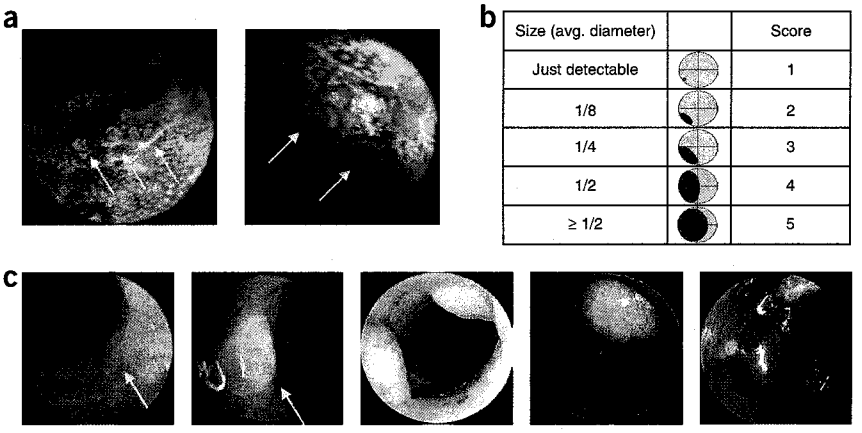

Size (avg. diameter)		Score
Just detectable		1
1/8		2
1/4		3
1/2		4
≥ 1/2		5

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Problems during endoscopy usually decrease with the experience of the investigator. Some of those problems are as follows:

Mortality of animals

Mortality of animals during the procedure can have several causes. First, it can be due to a perforation of the colon during routine endoscopy or upon taking of biopsies. Be careful not to push the endoscope with too much force if you feel resistance. Moreover, when taking biopsies, care should be taken to hold the biopsy forceps at a flat angle. Second, mortality can be due to the bad health status of the animals. Moribund animals with severe wasting are very sensitive to anesthesia and sometimes do not recover after endoscopy. Third, severe inflation of the gut during the procedure can harm the animals. If you observe marked inflation, immediately decrease the air flow of the pump.

Bad visibility

Bad visibility can be due to feces or insufficient air flow to inflate the colon. If feces is a problem, flush a small volume (100 µl) of saline into the colon using a Pasteur pipette. If feces or blood is coating the optical lens, withdraw the endoscope, clean it with water and reintroduce it.

ANTICIPATED RESULTS

We have developed a novel endoscopic procedure allowing high resolution imaging of the colon in live mice (**Fig. 2**). This method allows fascinating new insights into the pathogenesis of murine colitis and colon cancer by monitoring and scoring of colitis activity and colon cancer (**Figs. 3**, **4**). Furthermore, mouse colonoscopy permits colonic biopsies to be obtained from single tumors (**Fig. 5**). Since the investigator may analyze the success of the experiments early on and is able to monitor disease activity and extent *in vivo*, this approach may also help to limit the costs and the number of mice per experiment.

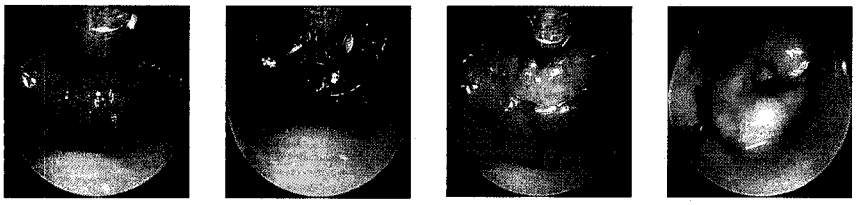

Figure 5 | Getting biopsies from the colon of a live mouse. Representative pictures taken during bioptic sampling of a tumor during routine endoscopic monitoring of a mouse. Tumor biopsies were snap frozen in liquid nitrogen and cut for staining.

Murine experimental models provide important tools to investigate the pathogenesis and therapies of chronic

intestinal inflammation and colon cancer in humans. The main advantages of using mouse models are the relatively easy breeding and the possibility of using syngeneic mouse strains. Furthermore, mouse lines transgenic or deficient for specific proteins provide important tools to investigate the role of these factors in colitis and colon cancer *in vivo*. However, an obstacle to the use of mice for investigating colon pathogenesis has always been the examination of the colon in live mice. Our endoscopic setup produced high resolution pictures of publication quality, allowing the degree of colitis and tumor development to be scored. Endoscopy was found to be safe, reproducible and fast, and usually took no longer than 2 min.

Using methylene blue to stain the colonic crypts, it is easily possible to detect lesions that were undetectable during standard colonoscopy without staining. Furthermore, preneoplastic aberrant crypt foci (ACFs) can be unmasked. Such chromoendoscopy allows the classification of the ACFs with regard to their pit pattern according to the Kudo classification[15].

We have performed more than 1,000 endoscopic examinations in mice, most of which had signs of severe colitis and multiple tumors. Only very few mice died after the endoscopy, most likely due to their inability to recover from anesthesia because of high disease activity and dramatic weight loss as a consequence thereof. Since most mice tolerated the procedure very well, mouse endoscopy can still be considered safe even for mice with very strong colitis and weight loss.

However, the protocol described here does have some limitations: the use of a rigid scope allows analysis of the distal half of the colon only. Although in most mouse models of colitis and colon cancer, the distal colon in particular is affected, other mouse models with pathology specifically in the proximal colon cannot be examined with the rigid scope described here due to the flexure of the colon.

Overall, we have developed a protocol for high resolution endoscopy, allowing the investigator to monitor the pathological changes on the mucosal surface of the colon and to take colon samples without killing the animal. Recently, the successful use of optical coherence tomography has been reported in mice[8,9]. Future use of the technique described here may combine mouse colonoscopy with fluorescence endoscopy and optical coherence tomography, thereby allowing molecular imaging and subsurface analysis of the mouse colon.

COMPETING INTERESTS STATEMENT The authors declare that they have no competing financial interests.

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1. Strober, W., Fuss, I.J. & Blumberg, R.S. The immunology of mucosal models of inflammation. *Annu. Rev. Immunol.* **20**, 495–549 (2002).
2. Boivin, G.P. *et al.* Pathology of mouse models of intestinal cancer: consensus report and recommendations. *Gastroenterology* **124**, 762–777 (2003).
3. Heijstek, M.W., Kranenburg, O. & Borel Rinkes, I.H. Mouse models of colorectal cancer and liver metastases. *Dig. Surg.* **22**, 16–25 (2005).
4. Wirtz, S. & Neurath, M.F. Animal models of intestinal inflammation: new insights into the molecular pathogenesis and immunotherapy of inflammatory bowel disease. *Int. J. Colorectal Dis.* **15**, 144–160 (2000).
5. Huang, E.H. *et al.* Colonoscopy in mice. *Surg. Endosc.* **16**, 22–24 (2002).
6. Wirtz, S., Becker, C., Blumberg, R., Galle, P.R. & Neurath, M.F. Treatment of T cell-dependent experimental colitis in SCID mice by local administration of an adenovirus expressing IL-18 antisense mRNA. *J. Immunol.* **168**, 411–420 (2002).
7. Funovics, M.A. *et al.* Miniaturized multichannel near infrared endoscope for mouse imaging. *Mol. Imaging* **2**, 350–357 (2003).
8. Tumlinson, A.R. *et al.* Endoscope-tip interferometer for ultrahigh resolution frequency domain optical coherence tomography in mouse colon. *Opt. Express* **14**, 1878–1887 (2006).
9. Hariri, L.P. *et al.* Endoscopic optical coherence tomography and laser-induced fluorescence spectroscopy in a murine colon cancer model. *Lasers Surg. Med.* **38**, 305–313 (2006).
10. Becker, C. *et al. In vivo* imaging of colitis and colon cancer development in mice using high resolution chromoendoscopy. *Gut* **54**, 950–954 (2005).
11. Becker, C. *et al.* IL-6 signaling promotes tumor growth in colorectal cancer. *Cell Cycle* **4**, 217–220 (2005).
12. Becker, C. *et al.* Cutting edge: IL-23 cross-regulates IL-12 production in T cell-dependent experimental colitis. *J. Immunol.* **177**, 2760–2764 (2006).
13. Huber, S. *et al.* Cutting edge: TGF-beta signaling is required for the *in vivo* expansion and immunosuppressive capacity of regulatory CD4+CD25+ T cells. *J. Immunol.* **173**, 6526–6531 (2004).
14. Becker, C. *et al.* TGF-beta suppresses tumor progression in colon cancer by inhibition of IL-6 trans-signaling. *Immunity* **21**, 491–501 (2004).
15. Kudo, S. *et al.* Colorectal tumours and pit pattern. *J. Clin. Pathol.* **47**, 880–885 (1994).