
Supplementary information

**High-speed light-sheet microscopy for the
in-situ acquisition of volumetric
histological images of living tissue**

In the format provided by the
authors and unedited

Supplementary Materials

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Supplementary Note 1: MediSCAPE design considerations and characterization

Basic principles of SCAPE microscopy

As illustrated in **Figure 1a**, swept confocally aligned planar excitation (SCAPE) microscopy illuminates the sample with an oblique light sheet, incident from a primary objective lens (O1). Fluorescence excited by this sheet is collected through the same objective lens. A lens telescope (TL1 and SL1) maps light between O1 and a galvanometer mirror which is used to sweep the excitation light-sheet from side to side in the sample, and to redirect the returning light into a second imaging telescope (TL2 and SL2) to a secondary objective lens (O2). This O2 lens creates an intermediate image of the sample, which remains stationary with respect to the scanning light sheet thanks to the descanning function of the galvanometer mirror. The oblique image of the light sheet in this intermediate image space is focused onto a camera by a third, obliquely aligned objective lens (O3) and tube lens (TL3) combination. An image splitter between O3 and TL3 can permit simultaneous imaging of two colours. This configuration permits images to be streamed to disk at the maximum rate from the system's camera, while the galvanometer is moved synchronously to scan the sample. Since each image acquired is an oblique y-z' depth section into the sample, it is not necessary to read out the entire camera chip because the number of rows on the camera corresponds to the number of depths. For samples requiring only ~100 camera rows, camera frame rates exceeding 2,000 frame per second (FPS) can be achieved, enabling 200 x-steps across the sample to be acquired at 10 volumes per second (VPS). A wide range of combinations of objective lenses can be used in this basic layout to optimize the system for different diffraction limited resolutions, 3D fields of view, imaging speeds and sample densities depending on the application¹⁻⁵. Here, in addition to our miniaturized MediSCAPE system (detailed below and in **Supplemental Note Table 2**), we used two different benchtop MediSCAPE prototypes (systems A and B detailed in **Supplemental Note Table 3** and **Supplemental Note Table 4**), with design **B** providing improved detection numerical aperture (NA) and throughput over a larger field of view than system **A**.

Advantages of single objective light sheet geometries

Standard, dual objective light sheet geometries illuminate through one objective lens and acquire through another oriented 90 degrees to the first⁶. This geometry limits the size and shape of samples that can be imaged because of the need for immersion media for high resolution imaging, precluding their use for imaging large, flat specimens and in vivo imaging of intact tissues. The volumetric imaging speed of dual objective systems is also limited by the need to change the position of the imaging plane in the sample, requiring either physical translation of the sample or system, or complex synchronization between scanning of the light sheet and the detection system's focal plane. SCAPE overcomes this challenge via its use of a single galvanometer mirror to both sweep its light sheet and descann returning light, maintaining the focus of camera on the moving light sheet with only this one moving part. SCAPE volumetric imaging can thus be achieved through a stationary objective lens without the need for physical restraint or motion of the sample making it ideal for in vivo, in situ imaging of intact and living tissues at speeds limited only by camera read-out rates and fluorophore brightness.

Advantages of light sheet illumination over point-scanning

Although SCAPE's scanning geometry permits very high-speed 3D image acquisition, obtaining high signal to noise with low phototoxicity is also essential. Confocal microendoscopy systems and most two-photon microscopy systems use point scanning, in which each individual pixel in the 3D tissue volume is excited and captured sequentially. Point-scanning thus severely limits available integration time per pixel,

requiring high laser powers while also requiring high galvanometer scanning speeds. MediSCAPE's use of light sheet excitation leads to significant improvements in sensitivity compared to point-scanning microscopy thanks to its parallelized excitation and detection of an entire plane in a tissue volume. This parallelization allows longer integration times and gentler laser excitation powers, which leads to reduced photobleaching and phototoxicity in tissues^{5,7}. **Supplemental Note Table 1** below shows the substantial difference between MediSCAPE's and a point-scanning microscope's galvanometer line scan rates and integration times per pixel for roughly equivalent volume imaging rates. Imaging parameters listed for MediSCAPE are from kidney datasets shown in **Figure 1** and **Supplementary Movie 6**, as an example.

Supplemental Note Table 1. | Comparison of imaging parameters on MediSCAPE and a point-scanning microscope for equivalent volume rates

	Volume Rate	FPS ³	X pixels	Y pixels	Z pixels	Galvo line scan rate	Pixel rate	Integration time per pixel
Stationary, high-resolution scan								
MediSCAPE¹	1.27 Hz	1021 Hz	802	615	250	1.27 Hz	157 MHz	0.98 ms
Point-scanning	1.27 Hz	317 Hz	802	615	250	195 kHz	157 MHz	6.3 ns
Roving, fast scan								
MediSCAPE²	9.3 Hz	1335 Hz	143	570	150	9.3 Hz	114 MHz	0.75 ms
Point-scanning	9.3 Hz	1400 Hz	143	570	150	200 kHz	114 MHz	8.8 ns

Notes:

1. Scan parameters based on mirror-based stationary scan of in vivo mouse kidney shown in **Figure 1 i-k**.
2. Scan parameters based on mirror-based roving scan of in vivo mouse kidney showed in **Supplementary Movie 6**.
3. FPS is calculated for yz frames for MediSCAPE and xy frames for point-scanning.

For a single high-resolution scan in the mouse kidney, MediSCAPE took 0.79 sec to acquire an $802 \times 615 \times 250$ xyz pixel volume of dual colour autofluorescence contrast. To acquire an equivalent single-color volume at the same speed, a point-scanning microscope would need to scan its galvanometer at a line rate of 195 kHz, a speed that cannot be achieved even using resonant scanners. Moreover, the integration time per pixel would be 6.3 ns, which is close to the fluorescent lifetime of many fluorophores. In contrast, MediSCAPE's scanning mirror would need to move at only 1.27 Hz, while the integration time per pixel would be 0.98 ms. This 153,379 times longer integration time highlights why weak intrinsic contrast can be imaged with MediSCAPE so much more easily than by confocal and two-photon while maintaining reasonable laser power levels and acquisition times (see Hillman et al for additional supporting models²). For a roving scan taken at 9.3 VPS, we see the same orders of magnitude difference between MediSCAPE and point scanning systems for the required galvanometer line scan rate and the resulting integration time per pixel.

Moreover, point-scanning microscopes designed for in vivo and bedside imaging require physical movement of internal lenses, the probe or tissue to acquire z-stacks. This can be both mechanically challenging to implement and prone to motion artifact in the presence of in vivo tissue movement. MediSCAPE's galvo-based lateral sheet scanning and simultaneous recording from all depths at once removes this need to vary the microscope's focal depth during 3D imaging. These differences in imaging speed and performance are demonstrated experimentally in **Supplementary Note 2**, which compares images acquired using MediSCAPE to confocal and two-photon point scanning.

Miniaturized MediSCAPE design details

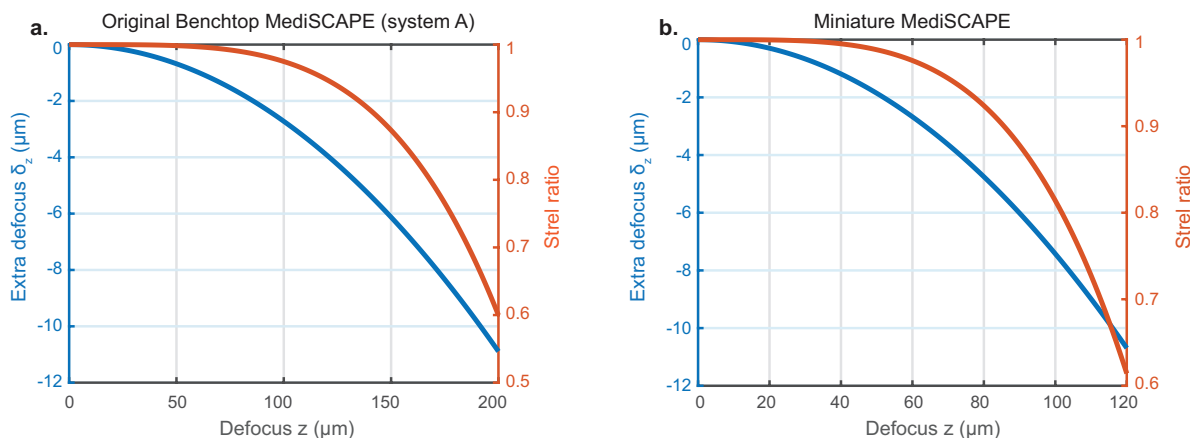
Based on imaging results obtained using benchtop MediSCAPE prototypes, we designed a more compact MediSCAPE microscope that is amenable to hand-held in vivo imaging, while maintaining cellular-level resolution and a practical depth range and lateral field of view. The overall miniaturized system schematic is illustrated in **Figure 1d**, with main components listed in **Supplemental Note Table 2**.

Major differences between the benchtop and miniaturized systems are the unfolded design and reduced sizes of lenses used in the compact system, and use of the system's intermediate image space to introduce the excitation light. Below, we detail our design considerations and choices of components for this system.

Primary & Secondary objectives: The primary objective (O1) should be chosen to provide as high NA and long working distance (WD) as possible. We replaced the 20x 1.0 NA Olympus XLUMPLFLN20XW used in our benchtop MediSCAPE system, which has a diameter of 30 mm, with the much smaller 60x Olympus water-immersion objective (LUMPLFLN 60XW), which is 22 mm in diameter and has a 3 mm effective focal length (EFL), 1.0 full NA and 2 mm WD in water. The primary impact of moving to this higher magnification objective is a reduction in the system's usable field of view (FOV) from 1.0 mm to ~0.6 mm.

To isotropically replicate the 3D sample volume imaged by O1 to the intermediate 3D image formed by O2, the semi-aperture acceptance angle of O2 should be no less than that of O1. Therefore, we chose as O2 a 50x Mitutoyo plano apochromatic objective (#58-237, Edmund) with 0.75 NA (in air) and a 4 mm EFL. This objective features a 5.2 mm WD, allowing sufficient space and flexibility both for re-imaging the stationary intermediate image through O3 and for launching of the excitation sheet.

Supplementary Note 1 Figure 1 shows the theoretical operational range of stigmatic imaging afforded by this O1-O2 combination⁸ as well as that of the typical benchtop MediSCAPE design. As shown, the O1-O2 combination can accommodate a defocusing range of about $\pm 80 \mu\text{m}$ (with Strehl ratio ≥ 0.9 and extra defocus less than $5 \mu\text{m}$) which provides a $\sim 160 \mu\text{m}$ axial range, sufficient for optical imaging of biological tissue.



Supplementary Note 1 Figure 1 | Operational range of stigmatic imaging for benchtop and miniature MediSCAPE systems. The Strehl ratio and coefficient of defocus of an on-axis point source at varying defocusing distances are calculated⁸ for **a**, the original benchtop MediSCAPE system where O1 = Olympus 20x 1.0 NA water objective (XLUMPLFLN20XW), O2 = Nikon 20x 0.75 NA objective (Plan Apo λ 20x), and **b**, the miniaturized MediSCAPE system where O1 = Olympus 60x 1.0 NA objective (LUMPLFLN60XW), O2 = Mitutoyo 50x plan apo objective (#58-237, Edmund). The O1-O2 combination chosen for MediSCAPE provides a good trade-off between operational range and compactness.

Relay telescopes: The scan and tube lenses (SL1 and TL1) between O1 to the galvo are chosen to meet the following criteria: 1) the outer diameter should be as compact as possible; 2) the entire 4f-system should form a long enough handheld portion for easy manoeuvrability; 3) the focal length of TL1 should be long enough to reach the back focal plane of O1, which is located 19.1 mm inside the objective, but not so long that there is cropping of marginal rays from the edges of O1's FOV (around 400 μ m in diameter); 4) the 6-mm-diameter back pupil of O1 should be de-magnified onto the galvanometer mirror without aperture loss. Factoring in these considerations, we chose to use a 12.7 mm diameter 38.1 mm EFL achromat as TL1 and a Plössl lens comprising two 12.7 mm diameter 50.8-mm-EFL achromats as SL1. The second relay telescope (SL2 and TL2) is situated further away from the sample space, so we relaxed their physical diameter constraint to 1 inch, choosing a Plössl lens made of two 1" diameter 101.6-mm-EFL achromats as SL2 and a 1" diameter 76.2-mm-EFL achromat as TL2. For all Plössl assemblies, separations between the constituent achromats were first modelled in OpticStudio 16.5 (Zemax LLC) and Solidworks 2016 (Dassault Systèmes), and then practically controlled with sub-mm precision by stacking optical spacers 0.4 mm or 1.0 mm in thickness (SM1S01, SM05S1M, and SM1S1M, Thorlabs).

Both of these telescopes have 1.5x magnification, but the ratio of the EFLs for O1 (3 mm) and O2 (4 mm) yields an effective magnification from the sample to the intermediate image of 1.33 $\times$, meeting the 'perfect 3D imaging condition' for re-imaging based on our use of water-immersion ($n = 1.33$) and air ($n = 1$) objectives for O1 and O2 respectively. The second telescope is folded close to the galvo mirror using a 90-degree silver mirror to form a linear configuration as shown in **Figure 1d-f**.

Excitation sheet launch: To further miniaturize this design, the location at which excitation light is launched into the system was moved from between SL2 and the galvo (as in our benchtop design shown in **Figure 1b**) to instead enter at O2, as previously described⁹. This approach effectively creates the light sheet at the intermediate image, and relays it to the sample in the same way as the returning image is relayed from the sample to the intermediate image. This approach reduces complexity of the imaging arm and moves the need for fine-tuned alignment to the more rigidly mounted part of the system.

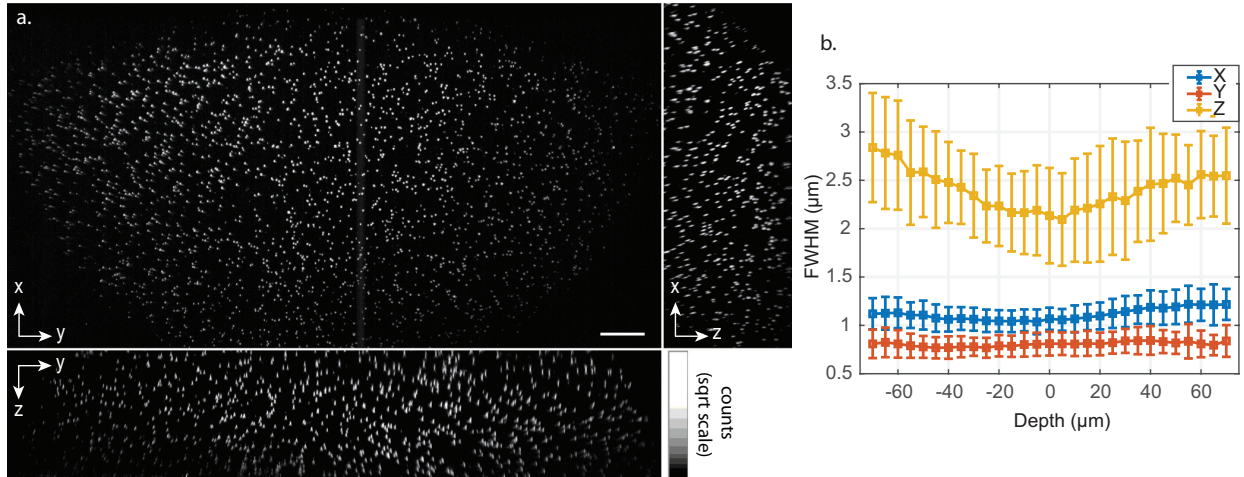
To form the sheet, laser light (488 nm) from a single-mode fibre (SM450, Thorlabs) with 2.8 μ m mode-field diameter was collimated by a 15 mm EFL aspheric lens into a Gaussian beam ~ 3.33 mm in $1/e^2$ waist. This Gaussian beam was expanded by $\sim 3.3\times$ by a cylindrical 4f-system (CL1 and CL2), and then focused by a 50-mm-EFL cylindrical lens (CL3), all along the x-direction, to generate an elliptical Gaussian beam. The beam waist of the sheet was carefully aligned to coincide with the focal plane of O2 and launched with a $\sim 39^\circ$ oblique angle into O2, corresponding to a ~ 2.5 mm lateral beam offset on the back aperture of O2. A slit in the light path permits adjustment of the light sheet NA at the sample and thus its thickness at its waist and range in Z over which the sheet remains narrow. 'Low NA' acquisition (NA ~ 0.06 -0.08) maximizes the depth range to around 300 microns while sacrificing x-direction resolution slightly, while 'High NA' (NA = 0.1-0.13) acquisition can provide finer resolution over a narrower depth range. We note that this system design is also compatible with implementation of non-Gaussian light sheet formation which could provide more uniform resolution if needed¹⁰.

Detection objective (O3): a Nikon Plan Apo λ 20 $\times$ 0.75NA objective was chosen as O3. It was paired with a tube lens (TL3) of appropriate focal length (e.g., 35 mm EFL for tissue imaging, or 135 mm EFL for resolution calibration) to magnify the intermediate image onto an sCMOS camera (Andor Zyla 4.2+). Since O3 is corrected for a 170 μ m thick coverslip, a coverslip mount was fabricated using 3D printing and installed in front of O3 to minimize spherical aberration.

Note that since O3 is oriented to focus on the oblique intermediate image formed by O2, it can only accept part of the emission cone collected by O1 and relayed to O2³. In general, the NA of O3 should be as high as possible, so long as it features enough working distance to avoid colliding with O2. For our choice of O3 and sheet angle of $\sim 39.5^\circ$, the equivalent system detection NA can be estimated to be 0.75 in Y and ~ 0.53 in Z (equivalent to a semi-collection angle of 23.3° in water). In comparison, the detection NA of benchtop SCAPE system **A** (O3 NA = 0.45; sheet angle = $\sim 37^\circ$; see **Supplemental Note Table 3**) is ~ 0.29 (equivalent to a semi-collection angle of $\sim 12.4^\circ$ in water) and the detection NA of the improved benchtop SCAPE **B** (O3 NA = 1.0 in water; sheet angle = $\sim 37^\circ$; see **Supplemental Note Table 4**) is ~ 0.50 (equivalent to a semi-collection angle of $\sim 22.1^\circ$ in water). The higher detection NA of the miniature MediSCAPE design is consistent with its relatively higher resolution and detection efficiency compared to benchtop system **A**.

System Resolution Characterization

Imaging properties of our basic benchtop MediSCAPE systems (**Supplemental Note Table 3**) match characterizations of SCAPE 2.0 designs provided in Voleti et al (Configuration D₂)³. For experimental characterization of our new miniaturized MediSCAPE design, fluorescent beads 200 nm in diameter embedded in 1% agarose gel were imaged for resolution characterization. A 135 mm EFL tube lens (SAL135F18Z, Sony) was used as TL3 to provide an overall $\sim 18\times$ magnification from the sample to the camera (Zyla 4.2+, 6.5 μm pixel size). Resulting images are shown in **Supplementary Note 2 Figure 3a**. Sampling densities were confirmed by manually translating the sample 100 μm along x-, y- or z-directions and quantifying bead displacements, yielding $\Delta_x = 0.337 \mu\text{m}$, $\Delta_y = 0.371 \mu\text{m}$, and $\Delta_z = 0.286 \mu\text{m}$ respectively. The sheet angle at the sample was calibrated to be 39.5° . After deskewing the MediSCAPE data, the FWHM resolution of the system was estimated to be $0.811 \pm 0.123 \mu\text{m}$ (y), $1.07 \pm 0.115 \mu\text{m}$ (x), and $2.10 \pm 0.479 \mu\text{m}$ (z) near the sheet waist (**Supplementary Note 2 Figure 3b**).



Supplementary Note 1 Figure 2 | MediSCAPE resolution characterization. **a.** Images of 200-nm fluorescent beads embedded in gel. Maximum intensity projections over 60 μm ranges are shown along all three axes from skew-corrected raw data. Each cross-section has been scaled to give an isotropic $\mu\text{m}/\text{pixel}$ over an xyz field of view of $390 \times 742 \times 145 \mu\text{m}^3$. Scale bar, 50 μm , square root grayscale for pixel counts to enhance contrast and show 3D field of view. **b.** Optical resolution is shown as the mean value $\pm$ STD of the FWHM of $n \sim 6,300$ total beads calculated as a function of z position. Beads were extracted from the skew-corrected three-dimensional data, and their FWHM size along all three directions were estimated. The beads were then grouped according to their depth into 5 μm thick intervals, for a total of 29 depths where the number of beads in each layer ranges from $n = 36 - 274$. Mean FWHM and standard deviation were calculated for each depth interval.

Although the x and y resolution do not change substantially with depth, the z-resolution worsens with distance from the beam waist, as expected based on the properties of the NA of the Gaussian light sheet (here used in ‘Low NA’ mode, ~ 0.08). Resolution could be improved at the centre by using a higher NA light sheet (at the expense of the usable depth range) or by better shaping the light sheet, incorporating beam waist scanning¹⁰. We also note that the resolution of MediSCAPE depends upon the combined effect of both the light sheet thickness and the detection NA such that resolution could be further improved by leveraging methods to maximize the effective collection angle of O3^{6,11}.

Mouse tissue and in vivo human imaging results

To compare this miniaturized system’s imaging performance to our benchtop MediSCAPE system, we imaged fresh, unstained mouse tissues as shown in **Figure 1m**, **Extended Data Figure 2** and **Supplementary Movie 5**. Using 488 nm excitation and ~ 4.6 mW incident power on the sample, we used galvanometer scanning to collect dual colour volumes of size $400 \times 700 \times 162 \mu\text{m}^3$ in xyz, acquired with a sampling density of $1.0 \times 1.4 \times 1.08 \mu\text{m}^3/\text{voxel}$ in xyz. Images were collected at 300 Hz (0.75 VPS) to compare tissue structures to those acquired with the benchtop system (shown in **Figure 1**, **Figure 2**, **Supplementary Movie 2** and **Supplementary Movie 3**). **Extended Data Figure 2a-d** shows cross-sections displaying tubules in the kidney cortex, the fibrous capsule and underlying cords of hepatocytes and sinusoids in the liver, cardiac muscle in the surface of the heart, and crypts of Lieberkühn in the colon mucosa. These volumes demonstrate very similar penetration depth and resolution of tissue structures compared to our benchtop MediSCAPE system (see **Figure 2**) albeit with a smaller, rounded field of view (caused by our use of a smaller form-factor 60x objective as O1). As a further demonstration of imaging performance in fresh tissue, we acquired $250 \times 700 \times 136 \mu\text{m}^3$ xyz volumes continuously at 11.2 VPS while roving across the colon mucosa. **Extended Data Figure 2e** shows a stitched field of view from 16 seconds of roving, created by pairwise stitching approximately every other volume collected, as described in the Methods in the main text. Insets to the right show clear crypt structure along each dimension, even with $2\mu\text{m}$ sampling density along x and frames collected at 1400 fps. During these scans, equivalent signal levels were detected on the Andor Zyla 4.2+ camera compared to our benchtop MediSCAPE system, consistent with models predicting that our miniaturized form of MediSCAPE actually collects a larger NA of emitted light, and is thus even more light-efficient than our benchtop system.

Figure 6 and **Supplementary Movie 15** show additional data acquired using the miniaturized MediSCAPE system in the human oral cavity in vivo, utilizing the objective cap shown in **Figure 1g** for tissue stabilization and spacing from the objective lens. Data is compared to data acquired using a benchtop MediSCAPE system with a larger field, confirming again equivalent levels of resolution, penetration depth and signal to noise, with a slight trade-off in field of view with the miniaturized system.

Further system miniaturization

This proof-of-concept miniaturized system was assembled using off the shelf components, achieving a form factor shown in **Figure 1e**. The resulting system performance confirms that MediSCAPE can be implemented using narrower lens telescopes and objective lenses, and can be configured in a much more compact form factor suitable for intraoperative use in open surgical fields as well as for oral and gynaecological examination.

Nevertheless, use of a smaller, custom primary objective lens (O1) could further reduce MediSCAPE’s imaging head diameter to potentially 4-6 mm¹²⁻¹⁵), enabling use in smaller orifices such as the ear, nose and throat. The galvanometer mirror in our current prototype can readily be replaced with a more compact

MEMS mirror¹⁶⁻¹⁸; especially since only a single-axis scanner is sufficient, allowing better compactness and robustness than dual axis scanners. Rod or GRIN lenses-based relay systems could be employed to extend the narrower part of the imaging arm to be more compatible with arthroscopy and laparoscopic insertion¹⁹, for example in combination with robotic surgery, while forward or side-facing MediSCAPE imaging could be implemented for intraluminal imaging.

As depicted in **Figure 1f**, the system's camera and laser sources could readily be located at a distance from the imaging head, relayed via fibre optic coupling if needed, while the whole unit could be mounted on a surgical microscope frame permitting flexible hand-guided movement of the imaging head within the surgical field, with small scale movements and scanning stabilized electromechanically.

Further miniaturization of MediSCAPE is also possible in principle, for example by combining fibre optic bundle-based detection with a proximal-end scan head using MEMs mirrors and GRIN lenses²⁰⁻²⁴. However, this implementation would likely sacrifice image quality and field of view, and would primarily serve gastroenterological endoscopy applications.

Supplemental Note Table 2 | Miniaturized MediSCAPE system component list

Part	Description	Part Number (Vendor)
Laser	Melles Griot DPSS 50 mW 488 nm	85-BDD-050-002
O1	Olympus 60× water objective, 2mm WD, 1.0NA W	LUMPLFLN 60XW (Olympus)
O2	Mitutoyo 50× plano apo HR objective, 5.2mm WD, 0.75NA	58-237 (Edmund Optics)
TL1	38.1 mm EFL achromat, 12.7mm dia.	49-775-INK (Edmund Optics)
SL1	Plössl lens: two 50.8 mm EFL achromats, 12.7mm dia.	49-777-INK (Edmund Optics)
Galvo	Galvanometer mirror with a 5-mm-diameter clear aperture	6210H (Cambridge)
SL2	Plössl lens: two 101.6 mm EFL achromats, 25.4mm dia.	49-783-INK (Edmund Optics)
TL2	76.2 mm EFL achromat, 25.4mm dia.	49-781-INK (Edmund Optics)
Asph	15 mm EFL aspheric collimator	F260FC-A (Thorlabs)
CL1	-15 mm EFL N-BK7 plano-concave cylindrical lens	LK1753L1-A (Thorlabs)
CL2	50 mm EFL N-BK7 mounted plano-convex cylindrical lens	LJ1695RM-A (Thorlabs)
CL3	50 mm EFL N-BK7 mounted plano-convex cylindrical lens	LJ1695RM-A (Thorlabs)
O3	Nikon plan apo λ 20×/0.75 objective, 1mm WD, 0.75NA	(Nikon)
TL3	35 mm EFL machine vision lens, f/1.4	MVL35M1 (Thorlabs)
	135 mm EFL, Sony Sonnar T*135mm F1.8 ZA lens	SAL135F18Z (Sony)
Camera	Scientific CMOS camera	Zyla 4.2+ (Andor Technology)

Supplemental Note Table 3 | Benchtop MediSCAPE system A component list

Part	Description	Part Number (Vendor)
Laser 1	Coherent OBIS LX 488 nm 150 mW	1220123 (Coherent)
Laser 2	Coherent OBIS LX 637 nm 140 mW	1196625 (Coherent)
O1	Olympus 20× water objective, 2mm WD, 1.0NA W	XLUMPLFLN20XW
O2	CFI Plan Apo λ 20×/0.75 objective, 1mm WD	MRD00205 (Nikon)
O3	CFI Plan Apo λ 10×/0.45 objective, 4mm WD	MRD70100 (Nikon)
TL1	150 mm EFL, VIS 0 coated achromatic lens, 50 mm dia.	49-285-INK (Edmund Optics)
SL1	Plössl lens (two 150 mm EFL, achromatic lens, 50 mm dia.	49-285-INK (Edmund Optics)
Galvo	1D Large Beam (10 mm) Galvo System, Silver-Coated Mirror	GVS011 (Thorlabs)
SL2	60 mm EFL, achromatic lens, 25.4 mm dia.	AC254-060-A (Thorlabs)
TL2	100 mm EFL, VIS 0 coated achromatic lens, 50 mm dia.	49-284-INK (Edmund Optics)
PL	Powell lens, 30° fan angle, 1mm beam width, 8.9mm dia.	LOCP-8.9R30-1.0 (Laserline)
CL1	50 mm EFL N-BK7 mounted plano-convex cylindrical lens	LJ1695RM-A (Thorlabs)
CL2	75 mm EFL N-BK7 mounted plano-convex cylindrical lens	LJ1703RM-A (Thorlabs)
TL3	0.35 Nikon digital adapter with c-mount: 0.7 digital adapter + 0.5 body tube / 70-200mm variable focal length tube lens	MQD42075 + MXA20714 (Nikon) / SAL135F18Z (Sony)
Camera	sCMOS camera	Zyla 4.2+ (Andor Technology)

Supplemental Note Table 4 | Benchtop MediSCAPE system B component list

Part	Description	Part Number (Vendor)
Laser1	Coherent OBIS LX 488 nm 150 mW	1220123 (Coherent)
Laser2	Coherent OBIS LX 660 nm 100 mW	1185057 (Coherent)
Laser3	Cobolt 06-DPL 561 nm 100 mW	0561-06-91-0100-100 (HÜBNER Photonics)
O1	Olympus 20× water objective, 2mm WD, 1.0NA W	XLUMPLFLN20XW
O2	Olympus 20× water objective, 2mm WD, 1.0NA W	XLUMPLFLN20XW
TL1	150mm EFL, VIS-NIR, achromatic lens, 50mm dia.	49-391-INK (Edmund Optics)
SL1	Plössl (2× 150mm EFL, achromatic lens, 50mm dia.)	49-391-INK (Edmund Optics)
Galvo	One axis dynAXIS 3S galvo with 10mm aperture.	124886 (SCANLAB)
SL2	Plössl (2× 150mm EFL, achromatic lens, 50mm dia.)	49-391-INK (Edmund Optics)
TL2	150mm EFL, VIS-NIR, achromatic lens, 50mm dia.	49-391-INK (Edmund Optics)
PL	Powell lens, 10° fan angle, 3mm beam width, 8.9mm dia.	LOCP-8.9R10-3.0 (Laserline)
CL1	75 mm EFL cylindrical achromat, 1" dia.	ACY254-075-A (Thorlabs)
CL2	100 mm EFL cylindrical achromat, 1" dia.	ACY254-100-A (Thorlabs)
O3	Olympus 20× water objective, 2mm WD, 1.0NA W	XLUMPLFLN20XW

*This system utilizes a custom-made water chamber between O2 and O3 to accommodate higher collection efficiency over a large field of view with 1.0 NA water immersion objectives throughout. This system also uses 2 cameras for dual colour acquisition.

Supplemental Note 1 References

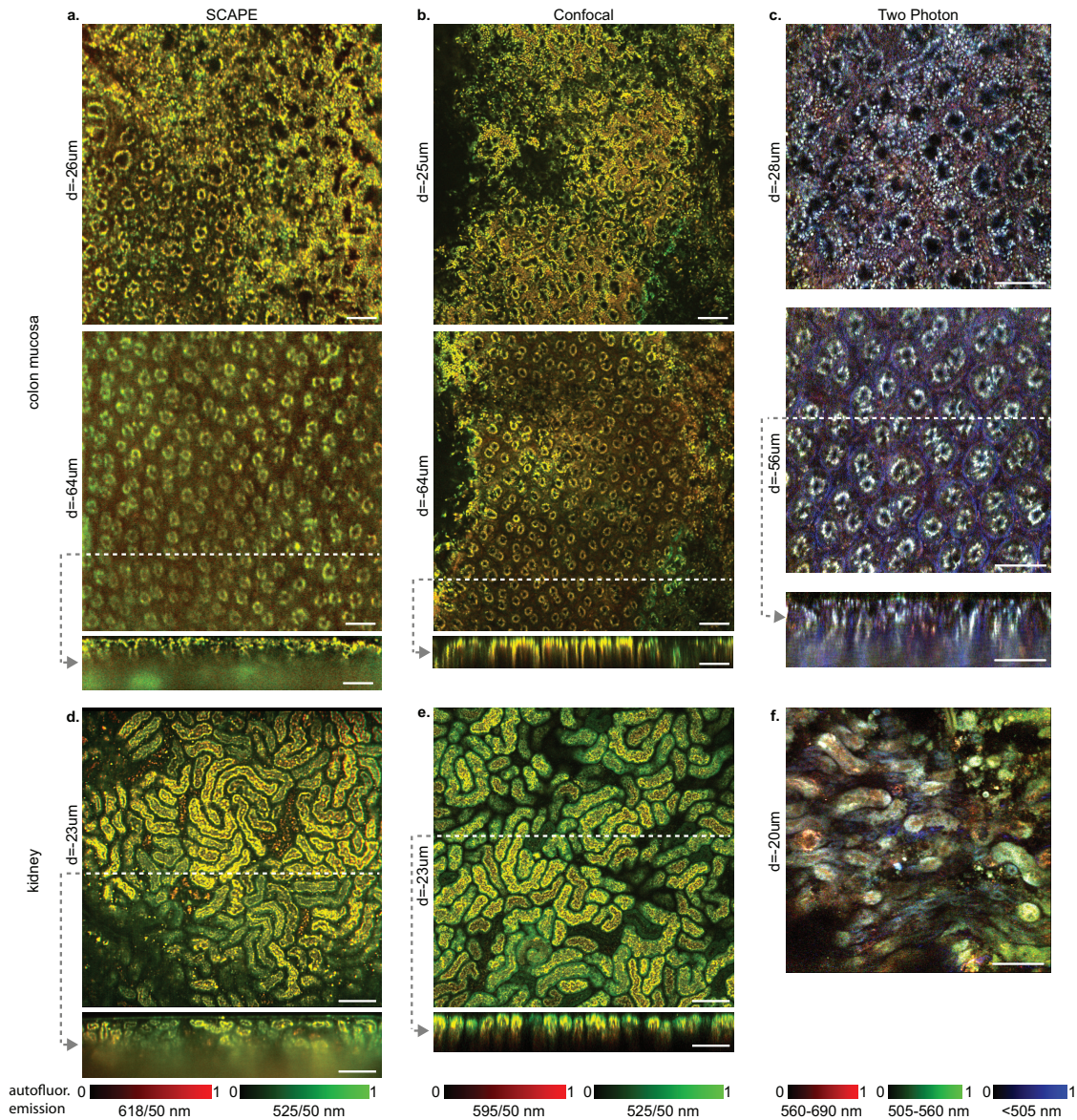
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Supplementary Note 2: Comparing MediSCAPE to confocal and two-photon microscopy

Comparison of autofluorescence contrast with MediSCAPE, confocal and two-photon

To compare autofluorescence features captured by MediSCAPE to other microscopy techniques, sections of freshly excised mouse colon mucosa and kidney cortex were imaged with confocal, two-photon and MediSCAPE microscopes consecutively. Confocal imaging was performed on a Nikon inverted A1R confocal. Two-photon imaging was performed on a previously described home-built system¹, using galvo-scanning and a Mai-Tai HP laser. Tissue was kept on ice between imaging sessions, kept moist with saline and imaged within three hours of excision.



Supplementary Note 2 Figure 1 | Autofluorescence visible with MediSCAPE, confocal and two-photon imaging. xy (top two panels) and yz (bottom panel) slices from volume stacks acquired in fresh mouse colon mucosa with **a**, MediSCAPE with 488nm excitation, **b**, confocal with 488nm and 561 nm excitation and **c**, two-photon microscopy with 800nm excitation. Dotted lines in xy slices indicated the location of yz cross-sections shown below. Autofluorescence was also imaged in fresh, intact mouse kidney cortex with **d**, MediSCAPE **e**, confocal and **f**, two-photon using the same excitation wavelengths as in the colon mucosa. Bottom panel indicates the bandpass filters used to separate each spectral channel shown. Imaging parameters for all stacks are summarized in **Supplemental Note Table 5**. All scale bars 100μm.

Representative images from each tissue are shown in **Supplementary Note 2 Figure 1** and imaging parameters used to acquire each volume shown are given in **Supplemental Note Table 5**. In fresh mouse colon mucosa, each imaging technique showed strong punctate green autofluorescence in the epithelial cells lining the crypts of Lieberkühn and diffuse red emission in the lamina propria in between crypt structures. Both MediSCAPE and confocal images were fairly similar, while two-photon excitation also revealed strong blue emission in the epithelial cells, as well as in fibres surrounding the crypts. In fresh mouse kidney cortex, all three imaging techniques were able to clearly visualize tubules through autofluorescence with proximal tubules showing higher emission at in the green channel and distal tubules showing higher emission in the red channel. Two-photon imaging also revealed blue autofluorescence in the tubules, overlapping highly with the green channel. Note that tubules here were imaged deeper into the kidney cortex and appear morphologically different from tubules closer to the cortex surface, as shown in MediSCAPE and confocal images. Also note that sample drift was a major issue in confocal and two-photon imaging over the course of volume acquisition in both tissues.

Although these datasets were acquired purely to compare contrast acquired on MediSCAPE to confocal and two-photon, imaging parameters shown in **Supplemental Note Table 5** show that confocal and two-photon imaging require almost two orders of magnitude more time to acquire a single-color volume of similar quality to images acquired using MediSCAPE in the same tissues.

Supplemental Note Table 5 | Imaging Parameters for SCAPE MediSCAPE, Confocal and Two-Photon

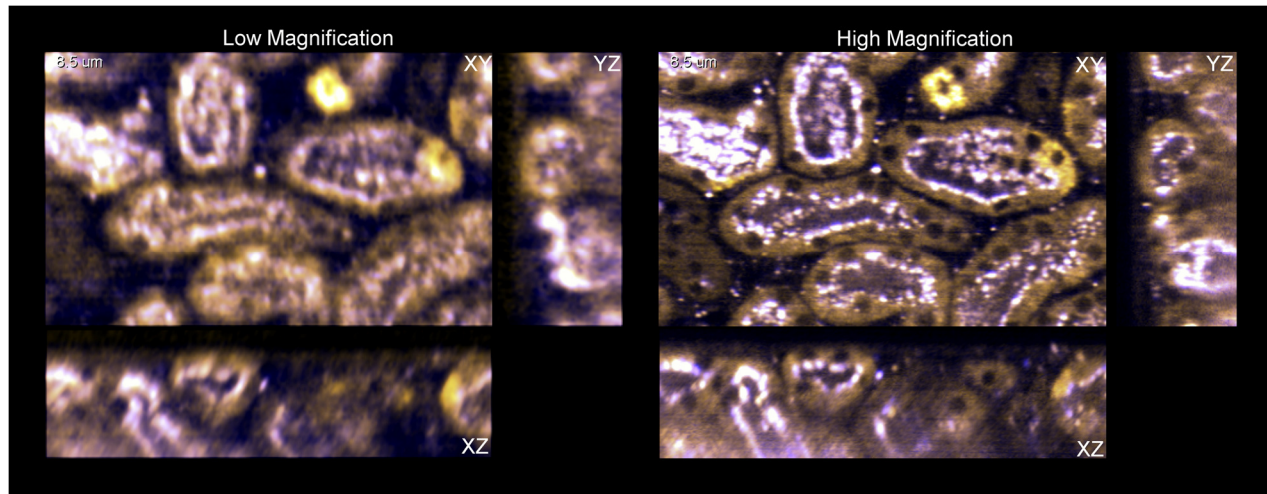
	SCAPE	Confocal	2P
NA	<0.45 (Nikon Plan Apo 10x + Olympus XLUMPLFLN 20X)	0.45 (Nikon Plan Apo 10x)	1.0 (Olympus XLUMPLFLN 20X)
Colon Mucosa			
Size (x-y-z μm^3)	991 x 1000 x 263	1228 x 1228 x 102	510 x 510 x 160
Size (x-y-z vox)	745 x 1000 x 240	1200 x 1200 x 37	600 x 600 x 80
# colour channels	2	2	3
Total Time (sec)	2	582	301
Voxel Rate*	179 MHz	183 kHz	287 kHz
Power at sample	7.5 mW (488 nm)	2.5 mW (488 nm) 3.8 mW (561 nm)	2.68 mW (800 nm, 80 MHz)
Kidney Cortex			
Size (x-y-z μm^3)	801 x 960 x 296	1228 x 1228 x 102	510 x 510 x 160
Size (x-y-z vox)	801 x 691 x 250	1024 x 1024 x 37	600 x 600 x 80
# colour channels	2	2	3
Total Time (sec)	8	286	301
Voxel Rate*	34.6 MHz	271 kHz	287 kHz
Power at sample	<6 mW (488 nm)	2.5 mW (488 nm) 3.8 mW (561 nm)	2.68 mW (800 nm, 80 MHz)

*Voxel rate calculated as # of colour voxels acquired per second of total imaging time
See **Supplementary Note 2 Figure 1** for corresponding images.

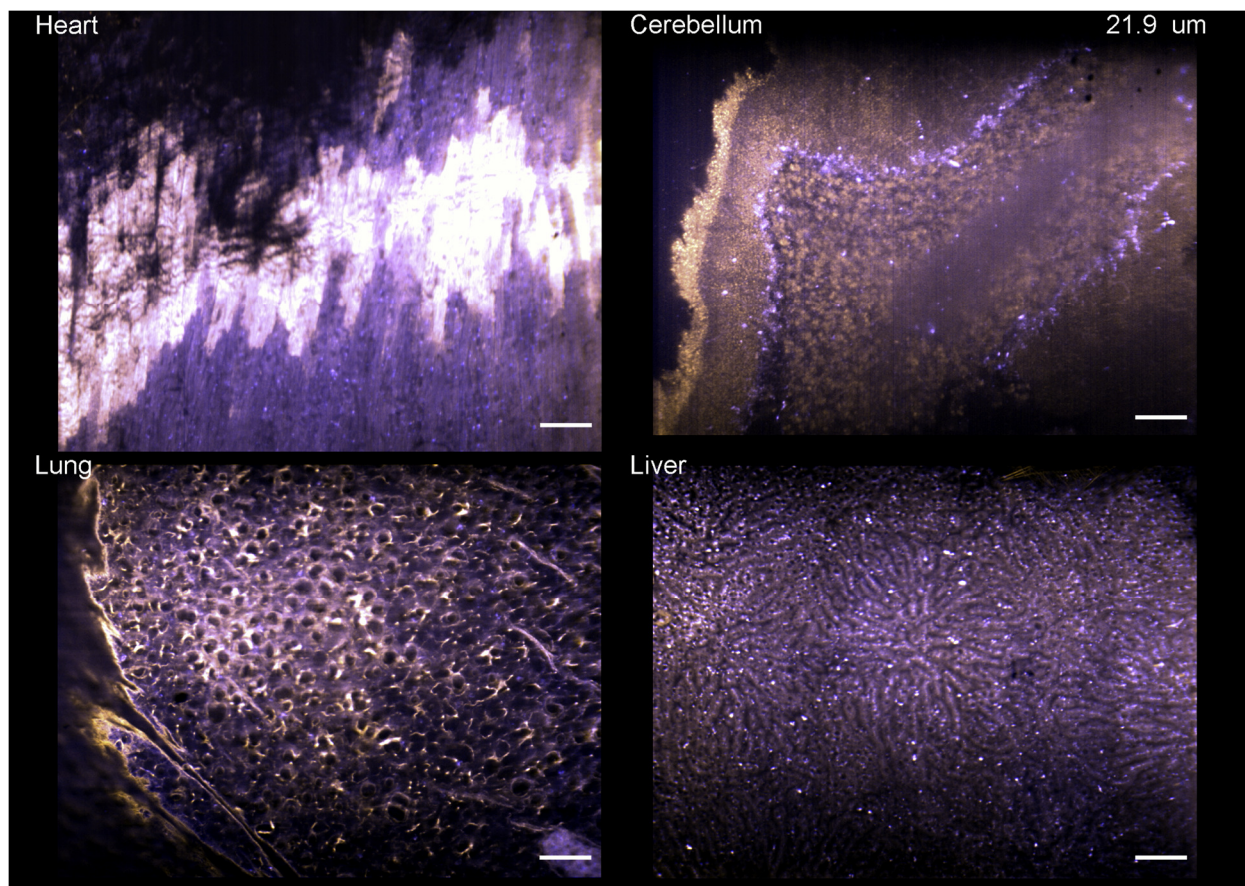
Supplemental Note 2 References

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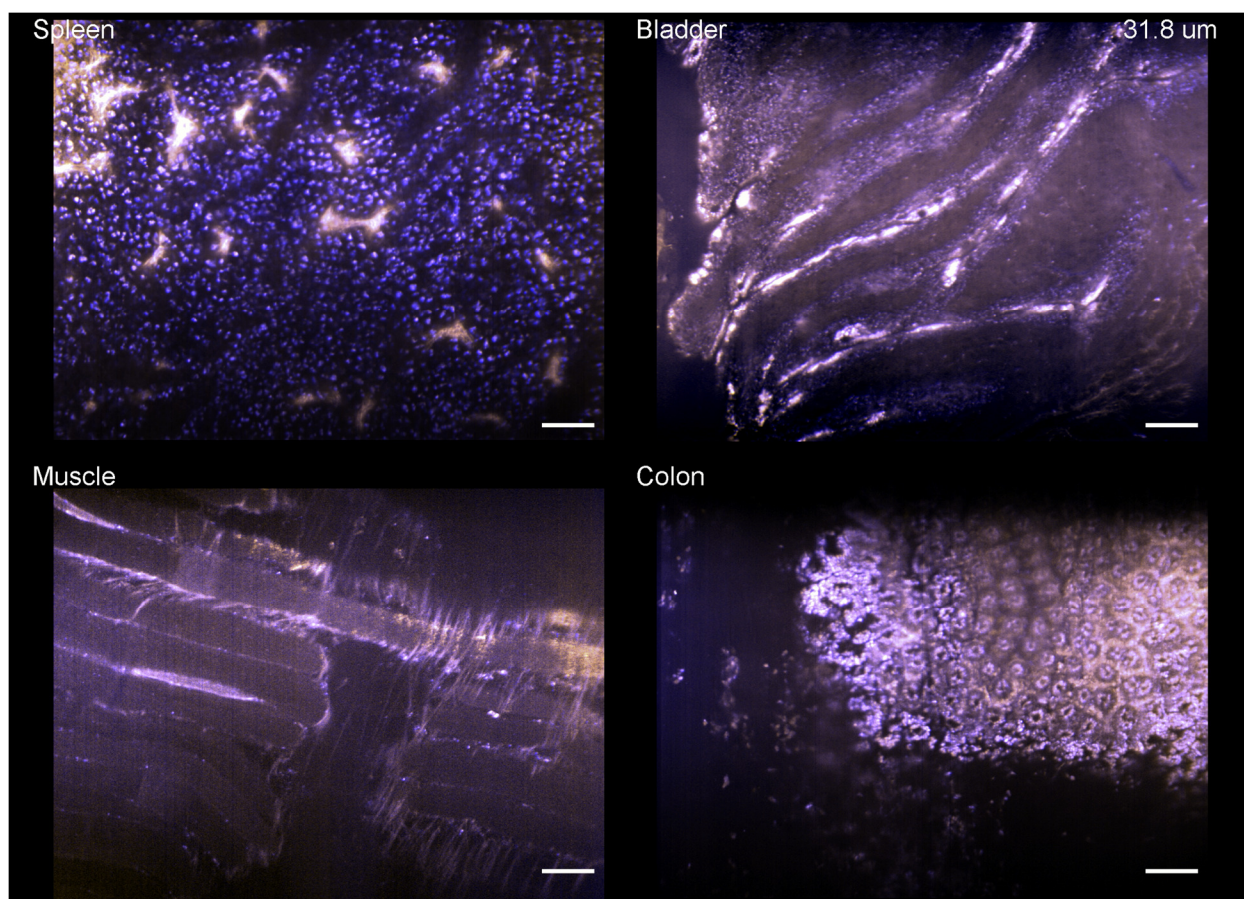
Supplementary Movies



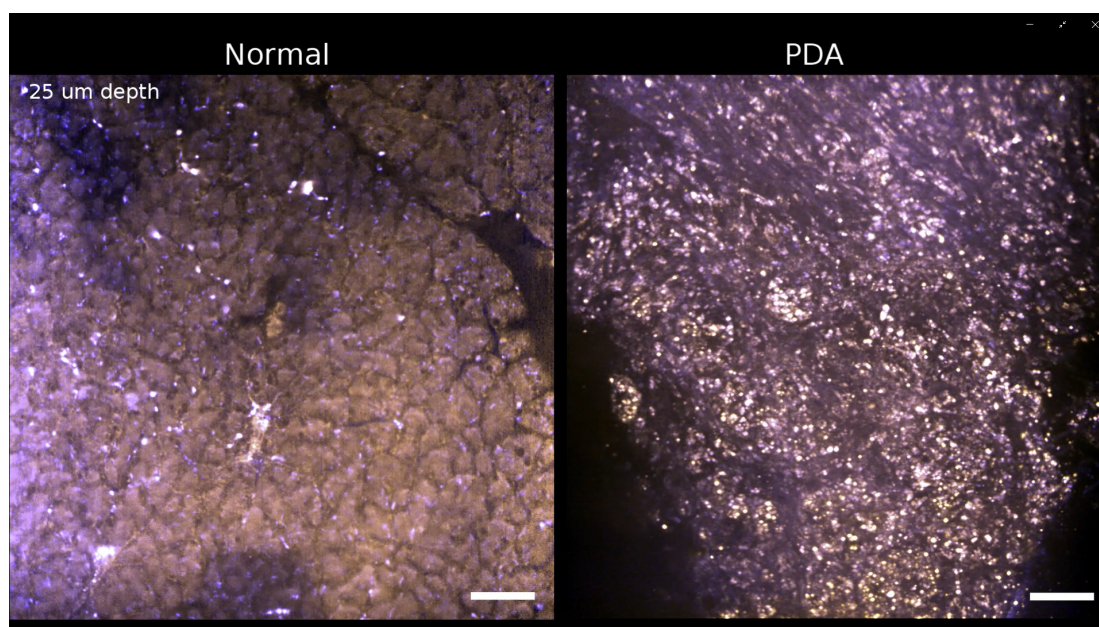
Supplementary Movie 1 | Fresh mouse kidney imaged with low and high magnification on MediSCAPE. Autofluorescence in the same region of fresh kidney tissue, imaged at different magnifications. A variable focal length tube lens allows dual colour imaging at a range of magnifications, adjustable based on the resolution, field of view and volume speeds required. Data on the left was collected with the variable tube lens set to $f=70$ mm for 4.6x magnification while data on the right was collected in the same region, immediately after setting the variable tube lens to $f = 170$ mm for 11.4x magnification. Volume stacks are slightly cropped to show the exact same region. Autofluorescence emission in the ~525nm range is shown in yellow hot, while emission at ~618nm is shown in blue. For full imaging parameters, see **Supplemental Table 1**. Scale bars, 100μm.



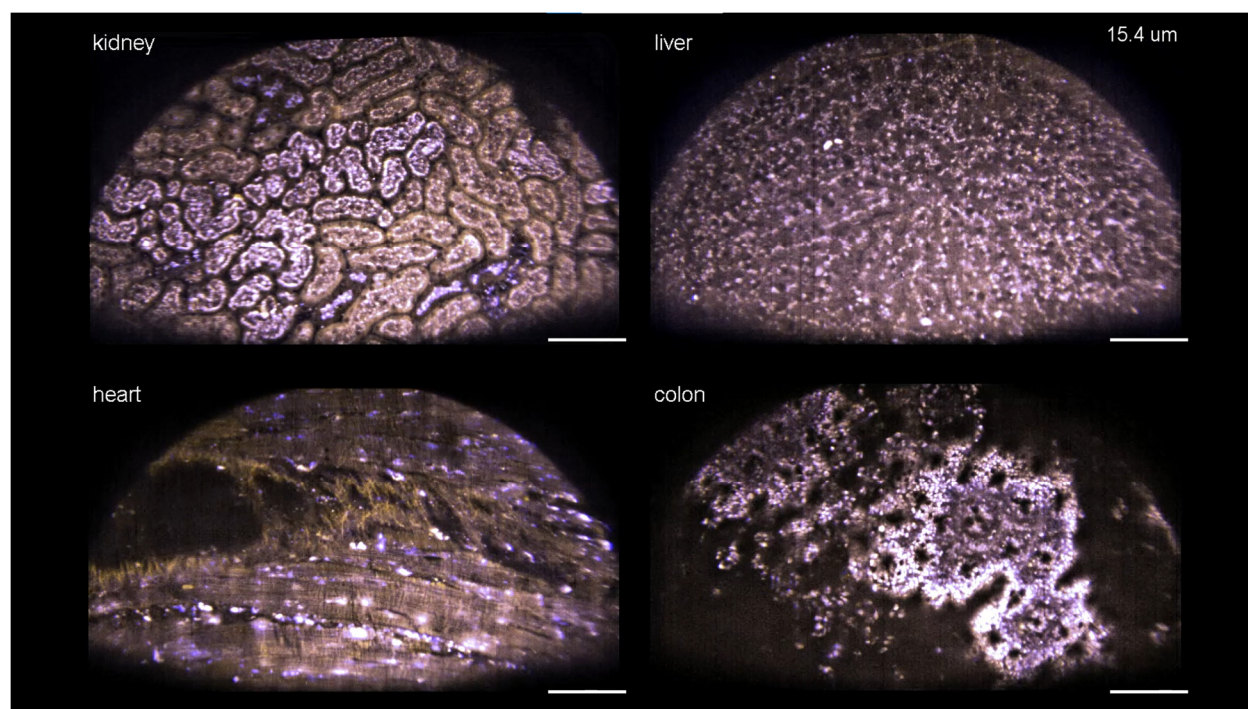
Supplementary Movie 2 | Autofluorescence in fresh mouse heart, brain, lung and liver imaged with MediSCAPE. Depth flythroughs of intrinsic contrast imaged in the first 50 μ m of intact, freshly excised mouse heart, sagittally-cut cerebellum, intact lung and intact liver tissue. For corresponding H&E histology, see **Figure 2a-d**. Autofluorescence emission in the ~525nm range is shown in yellow hot, while emission at ~618nm is shown in blue. For full imaging parameters, see **Supplemental Table 1**. All scale bars, 100 μ m.



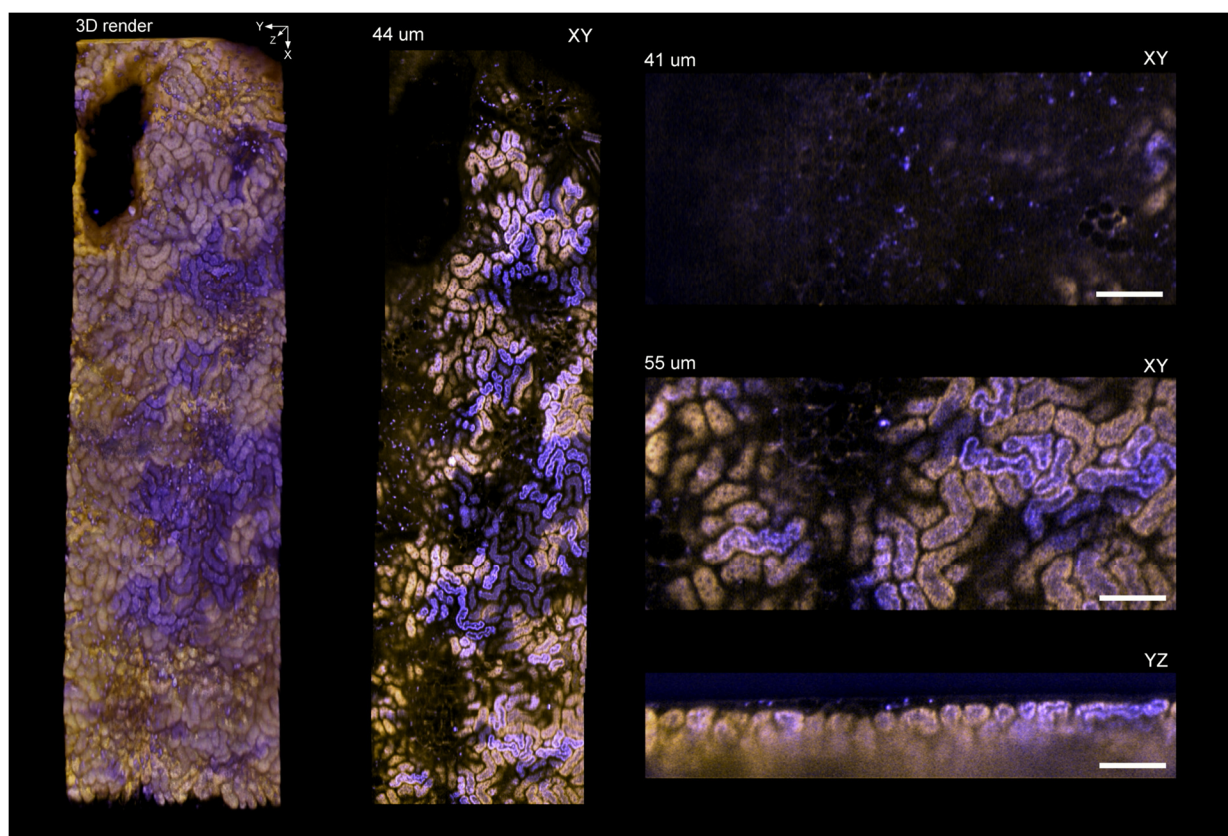
Supplementary Movie 3 | Autofluorescence in fresh mouse spleen, bladder, muscle and colon imaged with MediSCAPE. Depth flythroughs of intrinsic contrast imaged in the first 100 μ m of intact, freshly excised spleen surface, bladder mucosa, thigh muscle and colon mucosa. Autofluorescence emission in the $\sim$ 525nm range is shown in yellow hot, while emission at $\sim$ 618nm is shown in blue. For full imaging parameters, see **Supplemental Table 1**. For corresponding H&E histology, see **Figure 2e-h**. All scale bars, 100 μ m.



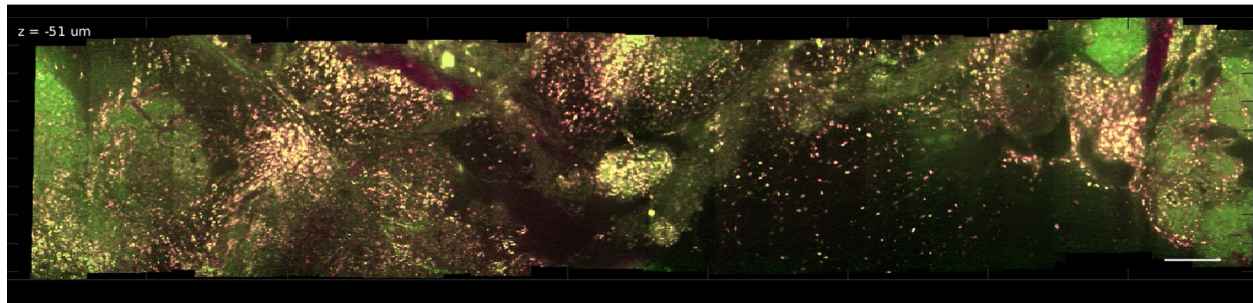
Supplementary Movie 4 | Autofluorescence in normal pancreas and pancreatic ductal adenocarcinoma (PDA) imaged with MediSCAPE. Depth flythroughs of intrinsic contrast imaged in the first 100 μ m of intact, freshly excised pancreatic tissue showing normal histoarchitecture (left) and cell distribution and cyst formation consistent with PDA. Autofluorescence emission in the ~525nm range is shown in yellow hot, while emission at ~618nm is shown in blue. For full imaging parameters, see **Supplemental Table 1**. For corresponding H&E histology, see **Figure 2i-j**. All scale bars, 100 μ m.



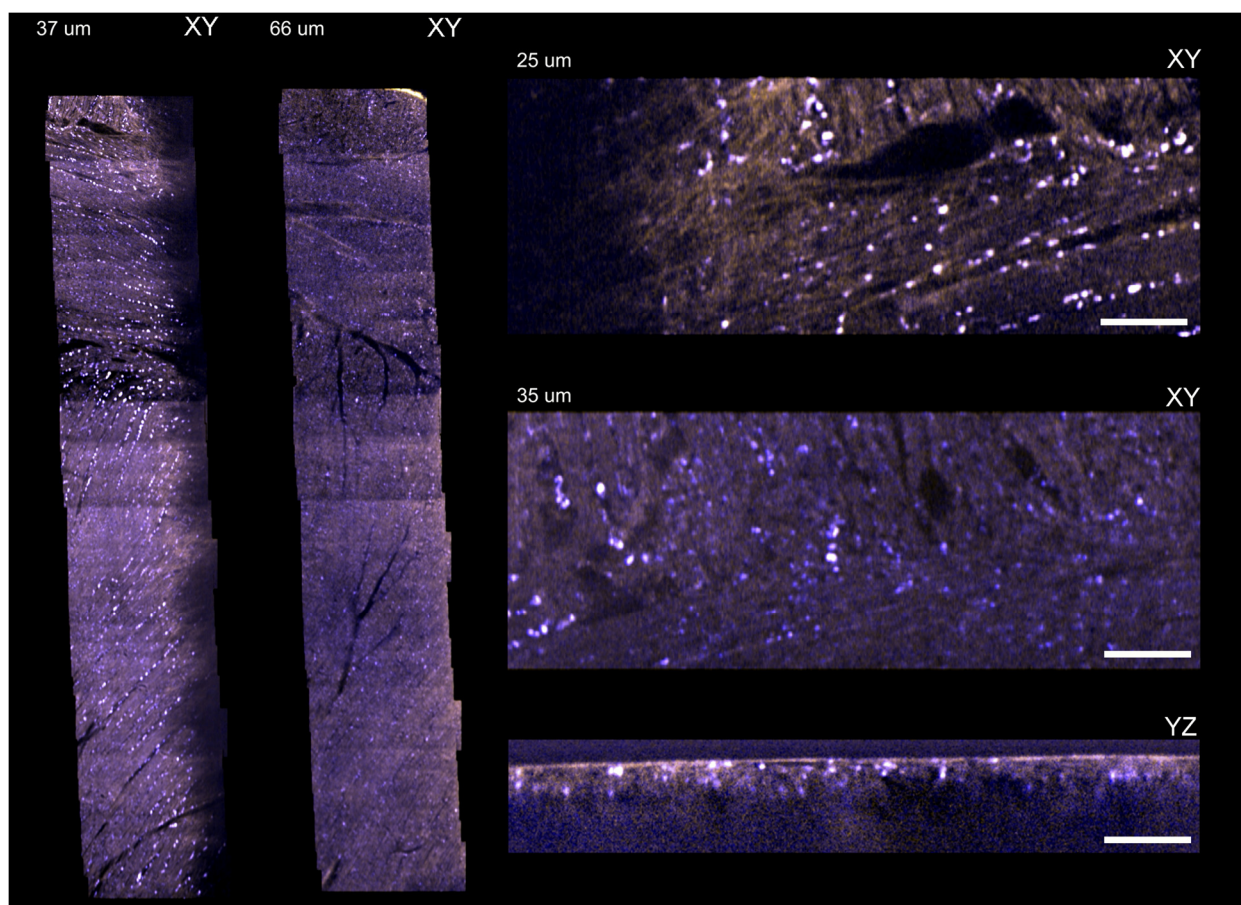
Supplementary Movie 5 | Fresh mouse kidney, liver, heart and colon imaged with a miniaturized version of MediSCAPE. Depth fly-throughs of intrinsic contrast imaged in the first 50 μ m of four types of fresh, intact mouse kidney, liver, heart and colon mucosa. Autofluorescence emission in the ~525nm range is shown in yellow hot, while emission at ~618nm is shown in blue. For full imaging parameters, see **Supplemental Table 1**. For corresponding single plane lateral and depth cross-sections see **Extended Data Figure 2**. All scale bars, 100 μ m.



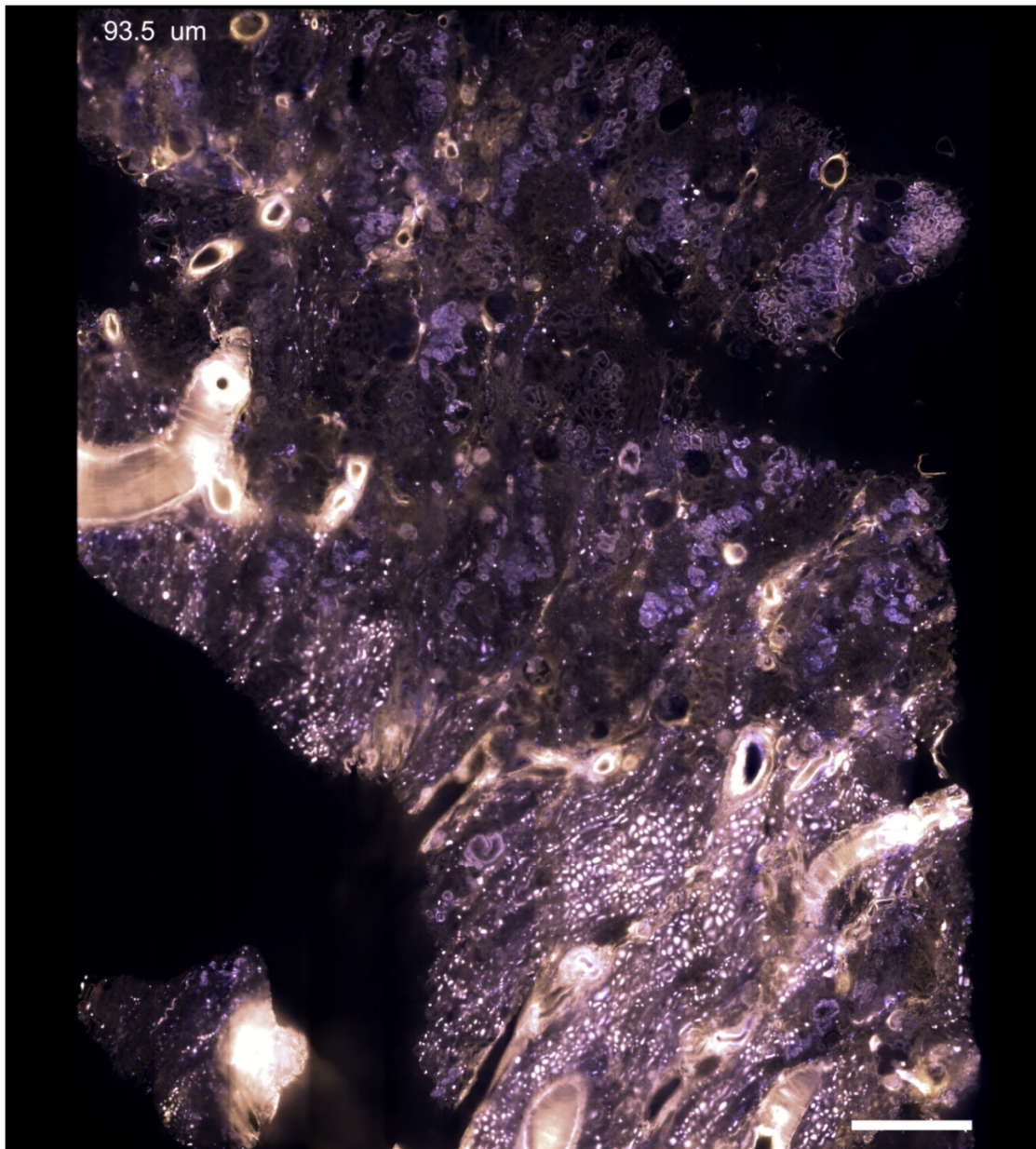
Supplementary Movie 6 | Label-free in vivo mouse kidney imaged with MediSCAPE at 9.3 VPS. Shown to the right are xy and yz cross-sections from $358 \times 798 \times 165 \mu\text{m}^3$ dual colour volumes collected at 9.3 VPS while roving across an in vivo mouse kidney. Shown to the left are a 3D rendering (ImageJ 3D Viewer) and a lateral cross-section of a larger field of view being stitched from overlapping volumes as they are collected. Playback is in real-time with stitching of volumes currently done in post-processing. The z depth of the xy plane shown is indicated on the top left of each plane. Autofluorescence emission in the $\sim 525\text{nm}$ range is shown in yellow hot, while emission at $\sim 618\text{nm}$ is shown in blue. For full imaging parameters, see **Supplemental Table 1**. All scale bars, $100\mu\text{m}$.



Supplementary Movie 7 | Stitched roving scan of in vivo label-free pancreatic tissue in a mouse model of pancreatic ductal adenocarcinoma (PDA) imaged with MediSCAPE. Shown is a depth-fly-through over 100 μm in depth. Volumes of size of $254 \times 920 \times 184 \mu\text{m}^3$ were collected in vivo at 10VPS over 24.9s to create the stitched volume. Autofluorescence emission in the $\sim 525\text{nm}$ range is shown in cyan-green, while emission at $\sim 618\text{nm}$ is shown in red-magenta. For full imaging parameters, see **Supplemental Table 1**. Scale bar, 200 μm .

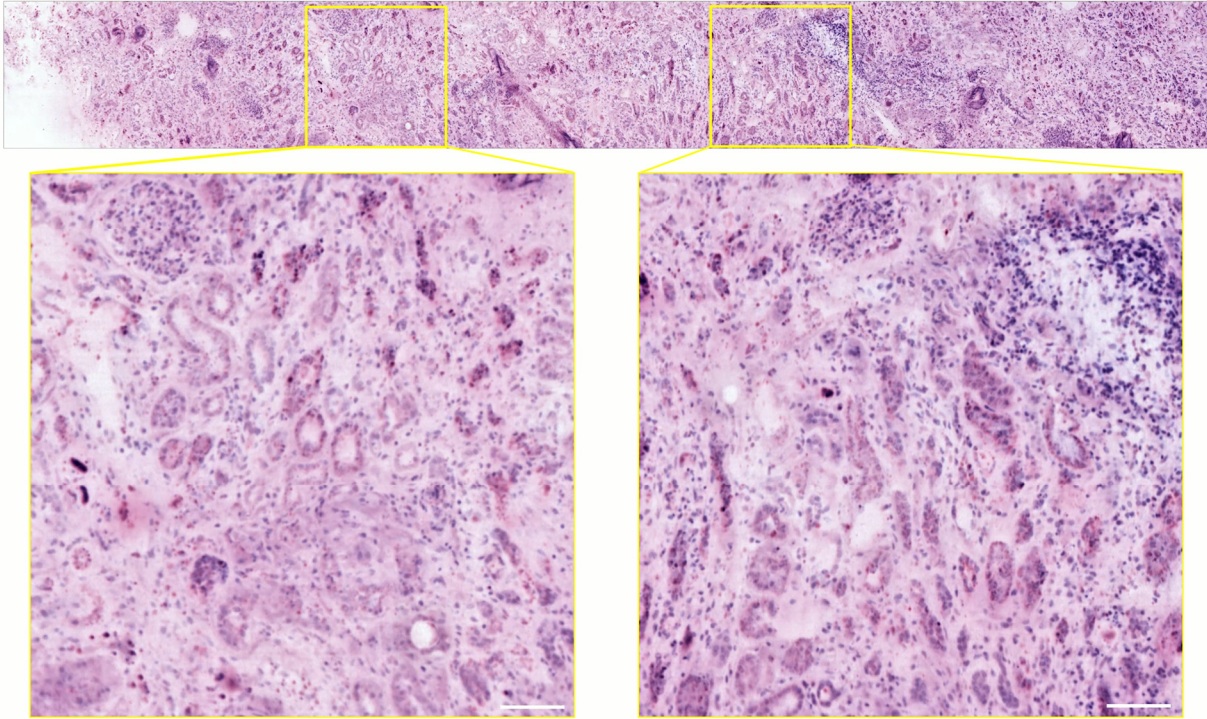


Supplementary Movie 8 | Label-free MediSCAPE imaging of in vivo mouse heart imaged at 12.9 VPS. Shown to the right are xy and yz cross-sections from $305 \times 798 \times 138 \mu\text{m}^3$ dual colour volumes collected at 12.9 VPS while roving across an intact, beating mouse heart. Cardiac pulses can be seen periodically in individual volumes and in the maximum intensity projection of data over time shown in **Figure 3**. Shown to the left are two lateral (xy) cross-sections at different z-depths from a larger 3D field of view being stitched from overlapping volumes as they are collected. Playback is in real-time with stitching of volumes currently done in post-processing. Autofluorescence emission in the $\sim 525\text{nm}$ range is shown in yellow hot, while emission at $\sim 618\text{nm}$ is shown in blue. For full imaging parameters, see **Supplemental Table 1**. All scale bars, $100\mu\text{m}$.

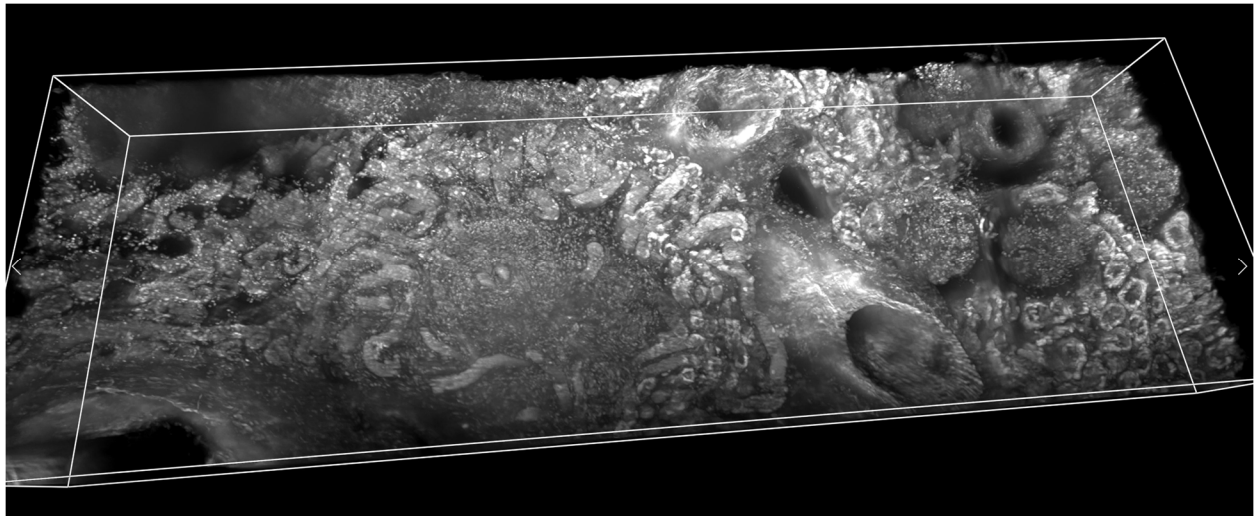


Supplementary Movie 9 | MediSCAPE autofluorescence image of fresh human kidney biopsy with chronic kidney disease. A $13.3 \times 10.6 \times 0.3 \text{ mm}^3$ xyz field of view was stitched from 12 stage-scanned dual colour volumes acquired in a total of 196 sec. Autofluorescence emission in the $\sim 525\text{nm}$ range is shown in yellow hot, while emission at $\sim 618\text{nm}$ is shown in blue. For full imaging parameters, see **Supplemental Table 1**. Diagnostic features within this volume are shown in **Figure 4** and **Figure 5**. Scale bars, $1000\mu\text{m}$ and $100\mu\text{m}$ (last, zoomed-in image).

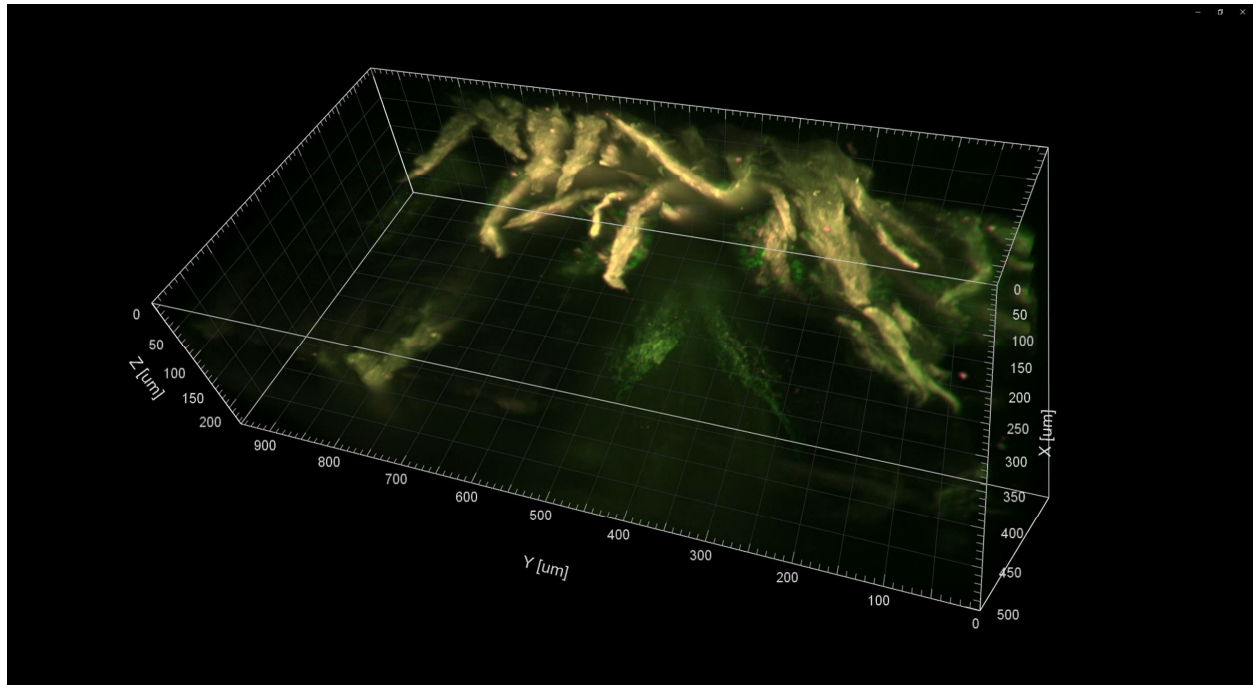
11.0 um



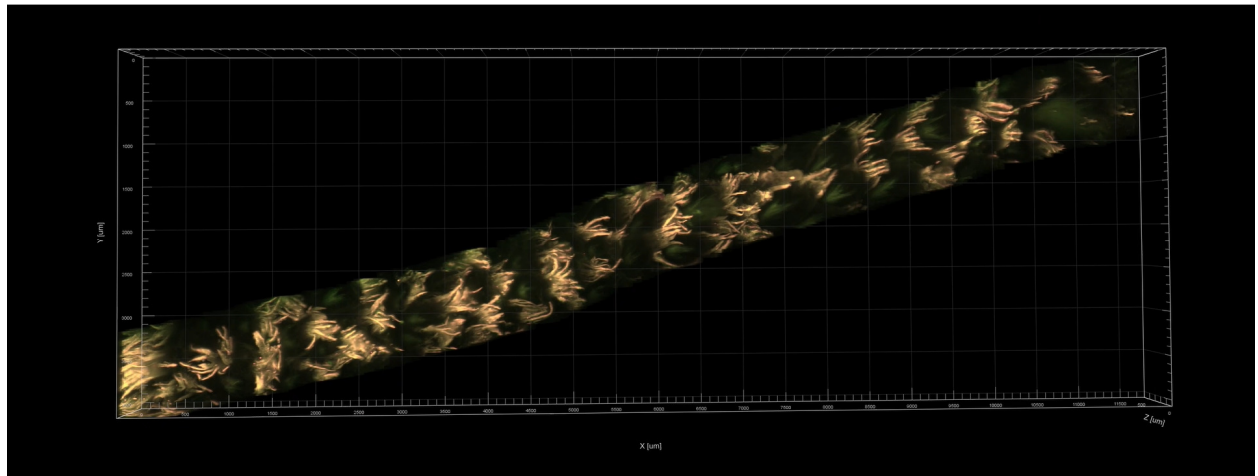
Supplementary Movie 10 | 3D H&E pseudocolour MediSCAPE image stack of fresh, normal human kidney tissue stained with proflavine. Proflavine fluorescence is encoded as haematoxylin (purple) and red autofluorescence emission encoded as eosin (pink) (488 nm excitation). Top panel shows the full 7500 x 918 x 164 μm^3 xyz volume, acquired by stage scanning in 5.6 s. Lower panels show smaller ROIs indicated. xy cross-sections are shown for the top 30 μm in depth (also shown in **Extended Data Figure 5**). All scale bars, 100 μm .



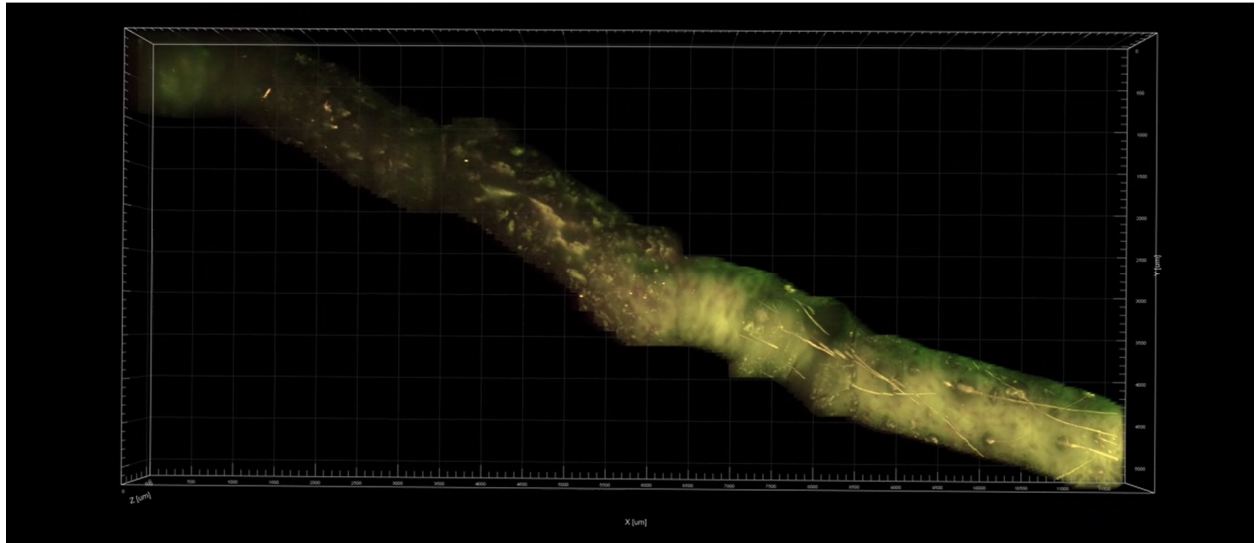
Supplementary Movie 11 | A 3D rendering and fly-through of proflavine-stained human kidney tissue imaged with MediSCAPE. A $2732 \times 921 \times 273 \text{ um}^3$ xyz stage-scanned volume from the same patient represented in **Extended Data Figure 6d-h**, showing signs of arterionephrosclerosis. A focal area of cortical scarring with tubular atrophy and interstitial fibrosis is evident near the centre. Glomeruli and arteries are clearly visible by autofluorescence and proflavine signals excited at 488nm, and their structures can be more easily evaluated by scrolling through both lateral and depth cross-sections. For more detailed discussion of histologic features visible, refer to **Extended Data Figure 6d-h**.



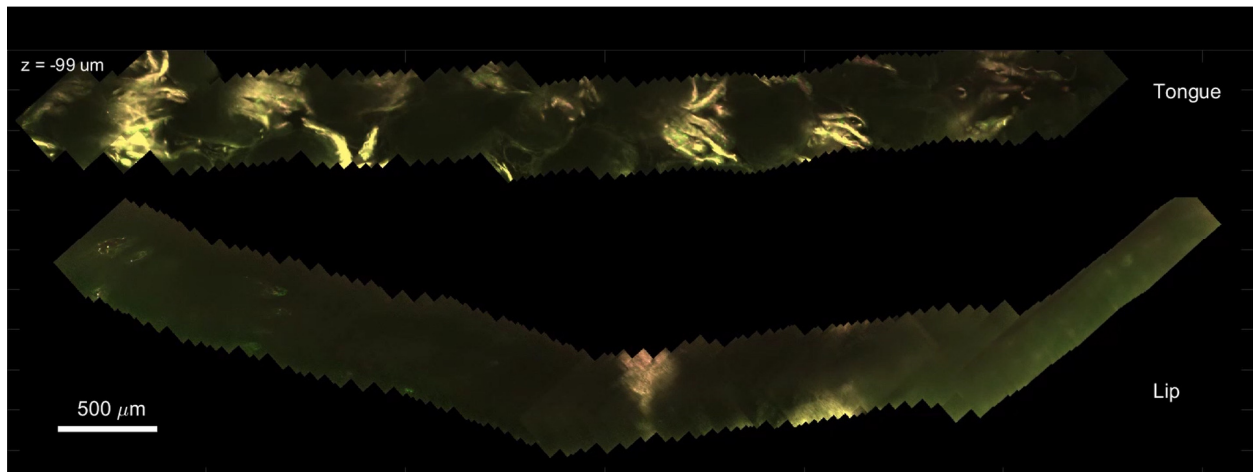
Supplementary Movie 12 | 3D rendering of MediSCAPE roving data acquired in-vivo on the human tongue. Raw-3D data shows the sequence of imaged volumes as a continuous movie acquired at 5VPS and shown at a 5fps playback speed. Filiform and fungiform papillae show strong and contrasting fluorescent characteristics. Rendering was performed in Imaris 9.5 (Bitplane Inc.). This dataset is part of **Figure 6a,c,d** with detailed imaging parameters given in **Supplemental Table 1**.



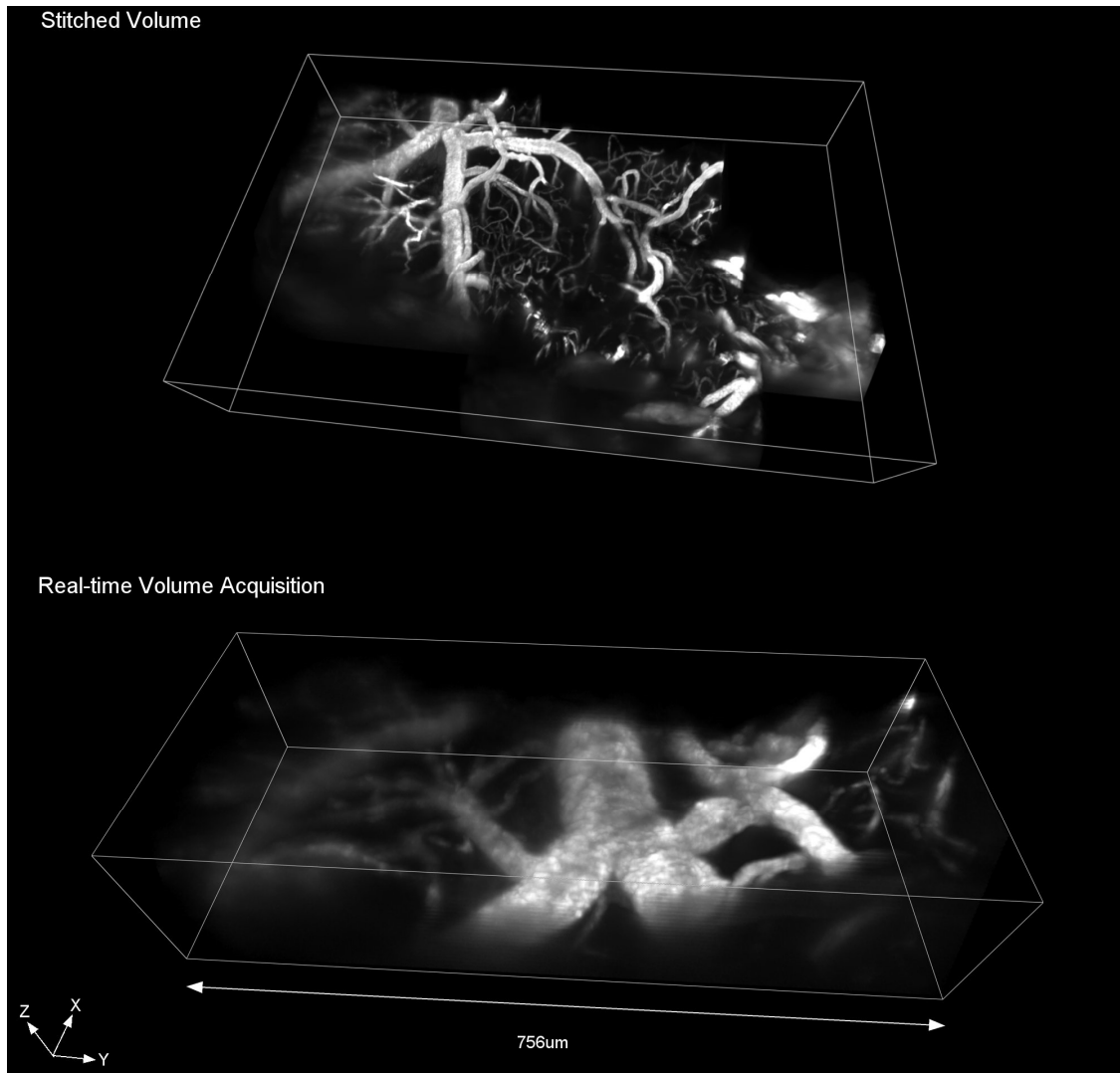
Supplementary Movie 13 | 3D rendering of stitched MediSCAPE roving data acquired in-vivo on the human tongue. Highlighting structural formation of filiform and fungiform papillae with contrasting fluorescent characteristics. Acquired with benchtop system B. Full length of strip = 11.8 mm. Rendering was performed in Imaris 9.5 (Bitplane Inc.) This dataset is part of **Figure 6a,c,d** with detailed imaging parameters given in **Supplemental Table 1**.



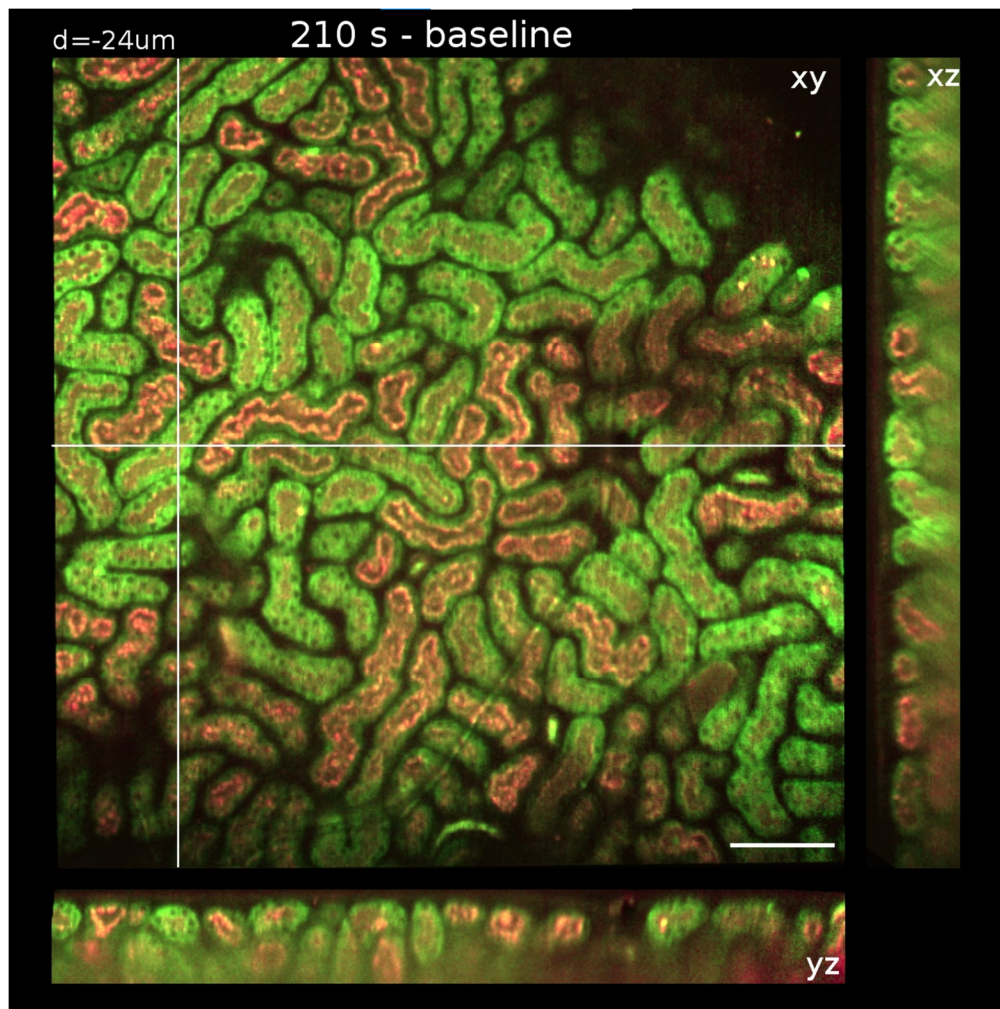
Supplementary Movie 14 | 3D rendering of MediSCAPE roving data acquired in-vivo on the human lip, roving from the inner lip (left) to the outer lip and skin (right). Highlighting the structural diversity of papillae morphology and showing follicles with surrounding, deeper capillaries in detail. Acquired with benchtop system B. Full length of strip = 11.6 mm. Rendering was performed in Imaris 9.5 (Bitplane Inc.) This dataset is part of **Figure 6 e,h,i,j** (bench) with detailed imaging parameters given in **Supplemental Table 1**



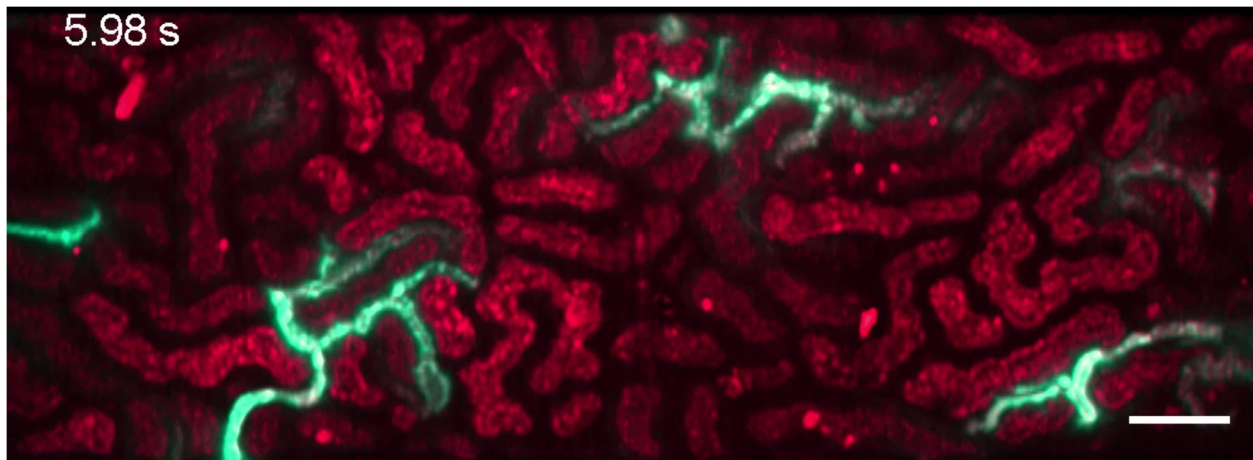
Supplementary Movie 15 | Depth fly-through of mini MediSCAPE roving data acquired in-vivo on the human tongue and inner lip, Acquired with the miniature MediSCAPE prototype. This dataset is part of **Figure 6c,d,g,h,i** (mini) with detailed imaging parameters given in **Supplemental Table 1**



Supplementary Movie 16 | Roving MediSCAPE imaging of in vivo mouse brain vasculature with IV FITC-dextran. Volumes were acquired through a glass cranial window at 9 VPS while roving around the cranial window. Bottom panel shows 3D rendering of real-time data during roving. Top panel shows rendering of a larger 3D field of view built up by stitching overlapping volumes as they are collected. Playback is in real-time with stitching of volumes currently done in post-processing.



Supplementary Movie 17 | In vivo time lapse of ischemia-reperfusion in mouse kidney acquired with MediSCAPE. Volumes of in vivo mouse kidney cortex were acquired every 30 seconds over a baseline, ischemia and reperfusion induced by clamping the renal vessels. White lines show the location of yz and xz depth sections. During the reperfusion period, white dotted lines show a local ischemic area as it is slowly reperfuses. Green autofluorescence is shown in green-cyan while red autofluorescence is shown in red-pink. Scale bars, 100 μ m



Supplementary Movie 18 | In vivo imaging of FNa infusion in mouse kidney in real-time with MediSCAPE. Volumes were acquired at 10 VPS over a 2 minute period to capture a fluorescein sodium bolus as it perfuses into the kidney cortex. Fluorescein sodium is shown in cyan while red autofluorescence is shown in red. Scale bar, 100 μ m.

Supplementary Tables

Supplemental Table 1 | Imaging parameters for MediSCAPE data.

Figure/Movie #	Panel/Sample ^a	Scan Type	SCAPE System ^b	xyz FOV (um ³) ^c	xyz Sampling Density (um ³ /vox) ^d	Frame Rate (Hz)	Volume Rate OR Total acq Time ^e	Laser Power @ Sample (mW) ^f
Fig 1 S. Movie 1	j-k; in vivo mouse kidney	1. mirror, stationary	A	802 x 861 x 275	1 x 1.4 x 1.1	1021	0.79s	7.5
	m(i); mouse kidney (left, low mag)	3. stage-scan	A*	500 x 436 x 275	1 x 1.4 x 1.1	496	16s	7.5
	m(ii); mouse kidney (right, high mag)	3. stage-scan	A*	500 x 487 x 225	0.5 x 0.56 x 0.45	50	40s	5
	n; mouse kidney	1. mirror, stationary	mini	400 x 700 x 162	1 x 1.4 x 1.08	300	1.3s	4.6
Fig 2, S. Movie 2-4	a, c-g; mouse heart, lung, liver, spleen, bladder, muscle	1. mirror, stationary	A	801 x 1065 x 330	1 x 1.4 x 1.1	100	8s	5
	b, h; mouse brain, colon	1. mirror, stationary	A	802 x 1065 x 275	1 x 1.4 x 1.1	100	8s	7.5
	i; normal pancreas	1. mirror, stationary	B	172 x 990 x 200	1 x 1.1 x 1	200	6s	8.5
	j; tumour pancreas	1. mirror, stationary	B	1162 x 972 x 300	1 x 1.1 x 1	100	12s	25.5
Fig 2, S. Movie 7, 8	d,f; inflamed pancreas	2. mirror, roving	B	single vol: 254 x 920 x 184 stitched: 4446 x 929 x 287	2.4 x 1.1 x 1	1050	10 VPS (24.9s)	25.5
	h-j; in vivo mouse heart	2. mirror, roving	A	single vol: 305 x 798 x 138 stitched: 4830 x 1102 x 185	2.5 x 1.4 x 1.1	1578	12.9 VPS (15.6s)	7.5
Fig 4, 5, S. Movie 9	Patient #1 (CKD), human kidney cortex tissue	3. stage-scan	A	12 strips: 13400 x 984 x 275, stitched: 13301 x 10629 x 309	1 x 1.4 x 1.1	814	196s	7.5
Fig 6 S. Movie 12, 13,14	a,c,d (bench); in vivo human tongue	2. mirror, roving	B	single vol: 506 x 1320 x 240 stitched: 11800x4070x500	2.98 x 1.1 x 1	850	5 VPS (30s)	16.9
	c,d (mini); in vivo human tongue	2. mirror, roving	mini	single vol: 350 x 558 x 229 stitched: 3852x4291x270	2.46 x 0.98 x 0.76	685	4.83 VPS (25s)	6.4
	e,h,i,j (bench); in vivo human lip	2. mirror, roving	B	single vol: 505.9 x 1320 x 400 stitched: 13106x5122x525	2.96 x 1.1 x 1	513	3 VPS (74s)	16.9
	g,h,i,j (mini); in vivo human lip	2. mirror, roving	mini	single vol: 350 x 558 x 305 stitched: 3753x3984x372	2.46 x 0.98 x 0.76	515	3.63 VPS (58s)	6.4
Fig 7 S. Movie 15	b, c; IV FITC-dextran, in vivo mouse brain	2. mirror, roving	A	single vol: 305 x 756 x 159 stitched: 1288 x 1880 x 258	2 x 1.21 x 1.06	1370	9 VPS (29s)	0.5
Fig 7 S. Movie 16, 17	e, f; ischemia, in vivo mouse kidney	1. mirror, stationary	B	1200 x 802 x 200	1 x 1.1 x 1	500	0.62 VPS	8.5
	g(i), g(ii); IV FNa, in vivo mouse kidney	1. mirror, stationary	B	1200 x 354 x 150	2.13 x 1 x 1	1328	8 VPS	1.7 (488nm)
	g(iii); IV FNa, in vivo mouse kidney	1. mirror, stationary	B	1200 x 1000 x 250	1 x 1.1 x 1	198	0.2 VPS	3 (561nm)
S. Fig 1	mouse brain	1. mirror, stationary	A	803 x 1061 x 275	1 x 1.4 x 1.1	200	4s	7.5
S. Fig 2, S. Movie 5	a-d; mouse kidney, liver, heart, colon	1. mirror, stationary	mini	400 x 700 x 162	1 x 1.4 x 1.08	300	1.3s	4.6
	e; mouse colon	2. mirror, roving	mini	single vol: 250 x 700 x 135 stitched: 4130 x 834 x 170	2 x 1.4 x 1.08	1407	11.2 VPS (16s)	4.6

S. Fig 3	Patient #3 (diabetic, hypertensive) kidney cortex tissue	3. stage-scan	A	4206 x 970 x 275	1 x 1.4 x 1.1	496	8.5s	5
S. Fig 4	Patient #2 (multicystic kidney), perirenal fat tissue	1. mirror, stationary	A	800 x 966 x 220	1 x 1.4 x 1.1	200	4s	7.5
S. Fig 5, S. Movie 10	Patient #2 (multicystic kidney), proflavine, normal cortex tissue	3. stage-scan	A	7500 x 918 x 164	1 x 1.4 x 1.1	1335	5.6s	7.5
S. Fig 6, S. Movie 11	a-c; Patient #4 (lumpectomy), proflavine normal tissue #1	3. stage-scan	A	4110 x 1121 x 274	1 x 1.4 x 1.1	407	10.1s	0.25
	d-h; Patient #4 (lumpectomy), proflavine normal tissue #1	3. stage-scan	A	2732 x 1121 x 274	1 x 1.4 x 1.1	407	6.7s	0.25
	i-k; Patient #4 (lumpectomy), methylene blue normal tissue #2	1. mirror, stationary	A	800 x 1121 x 165	1 x 1.4 x 1.1	777	1s	1 (637nm)
S. Fig 7	a, proflavine, mouse colon	1. mirror, stationary	A	800 x 1120 x 165	1 x 1.4 x 1.1	755	1s	0.1
	b, methylene blue, mouse colon c, fluorescein, mouse colon	1. mirror, stationary	A	800 x 971 x 165	1 x 1.4 x 1.1	789	1s	7.5 (637nm), 0.2 (488nm)
	d, mouse colon	1. mirror, stationary	A	801 x 1103 x 275	1 x 1.4 x 1.1	200	4s	7.5
S. Movie 6	in vivo mouse kidney	2. mirror, roving	A	single vol: 358 x 798 x 165 stitched: 2813 x 872 x 178	2.5 x 1.4 x 1.1	1335	9.3 VPS (11s)	7.5

Supplementary Table 1 Notes:

a. Samples were label-free fresh ex vivo tissue, unless otherwise noted.

b. **A** denotes the standard benchtop MediSCAPE system described in **Supplemental Note Table 3** (**A*** with alternate zoom lens as TL3). **B** denotes a second-generation benchtop MediSCAPE system described in **Supplemental Note Table 4**. ‘mini’ indicates the miniaturized version of MediSCAPE described in **Supplemental Note Table 2**.

c. For dual colour acquisitions, the y-dimension is given as the final cropped y-dimension of one-color channel. The original y-dimension on the camera is >2x the final cropped y-dimension because colour images are acquired simultaneously side-by-side along the y-axis on the camera. The x-dimension is given as the unskewed x-dimension of the acquired volume (# x-steps * x-step size).

d. Scans were acquired with a 70mm focal length tube lens giving 4.66x effective magnification, unless otherwise noted.

e. Volume rate is reported if the scan type is a mirror-based roving scan. For mirror-based stationery and stage-scans the total acquisition type is given in seconds. For stitched roving scans, the total time to acquire the volumes stitched is given in parentheses.

f. Laser power is for 488nm laser excitation, except for **Extended Data Figure 5j** and **Extended Data Figure 6b** where 637nm excitation was used for methylene blue and **Figure 7g** where 561nm laser light was used for excitation of red autofluorescence.

Supplementary Table 2 | Intrinsic fluorescence sources in biological tissues.

Source of Intrinsic Fluorescence		Notes	Excitation (nm)	Emission (nm)	Citing Literature
Structural proteins					
Collagen fibres		Found in arteries, liver/kidney capsule, lungs, skin, connective tissue, areas of fibrosis; ~1-20µm thick, wavy strands composed of 30-100 nm thick fibrils; fluoresces when cross-linked	335, 460	380, 440, 520	1-3
Elastin fibres		Found in arteries, liver/kidney capsule, lungs, skin, connective tissue; cord or sheet composed of ~0.1-0.2µm thick fibrils; fluorescence associated with cross-links	340, 460	440, 520	1-3
Keratin		Found in keratinized epithelium (hair, skin, etc.); intracellular fibrous protein; collagen-like excitation and emission	280-325	460	4,5
Lipids					
Lipo-pigments	Lipofuscin	Found in brain, heart, muscle, liver, etc.; granular, strongly fluorescent; product of normal aging + oxidative stress; involved in age-related diseases like Alzheimer's, Parkinson's and heart failure	320-480	460-630	6,7
	Ceroids	Granular, strongly fluorescent. Involved in neurodegenerative diseases, nutritional cirrhosis, Vitamin E deficiency, atherosclerosis	340, 460	440, 520	6,8
Phospholipids		----	436	540, 560	9,10
Fatty acids		Previously imaged in liver; includes arachidonic, palmitic, linoleic and oleic acid	~366	415-540	11
Enzymes involved in Cellular Metabolism					
Flavins	FAD	Flavin adenine dinucleotide, oxidized form; changes in fluorescence due to concentration or binding state. Responsible for intense autofluorescence in eosinophils ¹²	375, 450	525	9,13,14
	Riboflavin	Vitamin B ₂	480-490	530-540	14,15
	FMN	Flavin mononucleotide	373, 445	525	13,14
NAD(P)H		Nicotinamide adenine dinucleotide, reduced form; changes in fluorescence due to concentration or binding state	290, 351	440, 460	9,16,17
Other enzymes and coenzymes					
Retinol		Vitamin A ₁ ; imaged in liver, blood, eyes; levels can depend on oxidative stress; involved in vision health	325	520	11,18
Cholecalciferol		Vitamin D	390	480	9
Vitamin K		----	335	480	9
Vitamin B ₆		----	315-340	385-425	19
Folic Acid		Vitamin B ₉	400-449	485	20
Vitamin B ₁₂		----	275	305	19
Aromatic Amino Acids					
Tryptophan		All contribute to protein fluorescence; phenylalanine quantum yield relatively low; tryptophan's indoleamine derivatives (serotonin and melatonin) also fluorescent	295	353	16,21,22
Tyrosine			275	353	16,21
Phenylalanine			260	282	16,21

Other

Porphyrins	Protoporphyrin IX	Increased levels in tumour cells + skin with acne vulgaris; involved in heme synthesis; production stimulated by exogenous 5-ALA; sensitive to photobleaching	407, 488	630-700	4,23
Purines, pyrimidines and nucleic acids		Adenine, adenosine, guanine, guanosine, ADP, ATP, RNA, DNA	260-285	340-390	10,24
Bilirubin		Yellow pigment found in bile in the liver; results from normal heme catabolism	366, 465	520, 570	25
Hyaline		Descriptor; hyaline deposits/casts or hyalinized tissues appear glassy and are found in some sclerotic glomeruli, the optic disc and vasculature	470-490	~515	26,27
AGEs (Advanced Glycation End-Products)		Found in the skin and eye; results from oxidative and glycaemic stress; increased levels in diabetes, renal disease and cardiovascular disease; formed on proteins from non-enzymatic glycation or oxidative reactions	350-390	420-600	28,29
Melanin		Pigment found in melanocytes in skin, hair and eyes; weak fluorescence under specific conditions at UV excitation; strongly absorbing	400-440, 785	~580, >800	30-32

Supplementary Table 2 Notes:

Fluorophores highlighted in grey are likely visible under 488 nm excitation in MediSCAPE. The ~525 nm emission channel likely captures elastin, flavins, lipofuscin, ceroids, phospholipids, bilirubin and hyaline while the ~618 nm channel captures relatively more signal from lipofuscin, ceroids and porphyrins.

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