# Supplementary Information for

## A clinically translatable hyperspectral endoscopy (HySE) system for imaging the gastrointestinal tract

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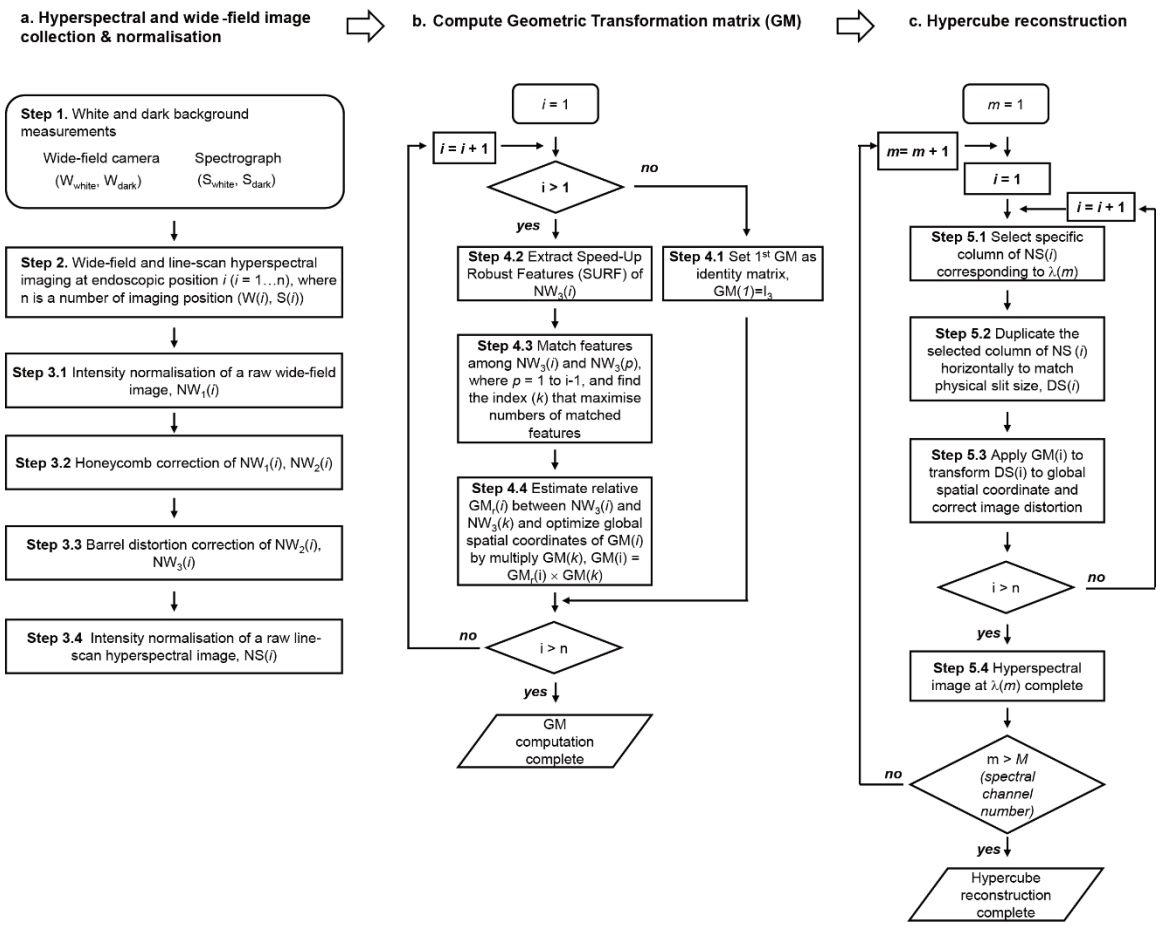
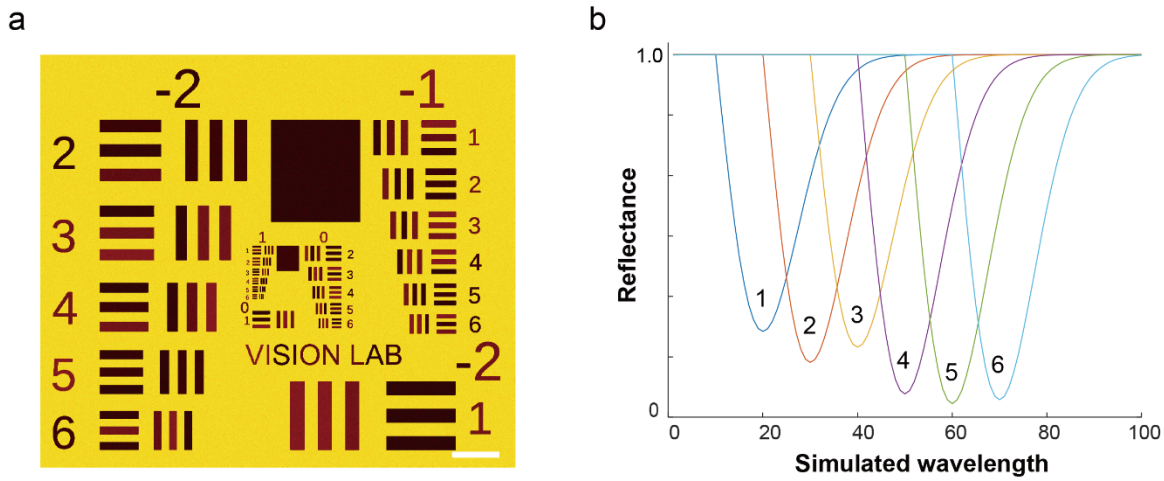
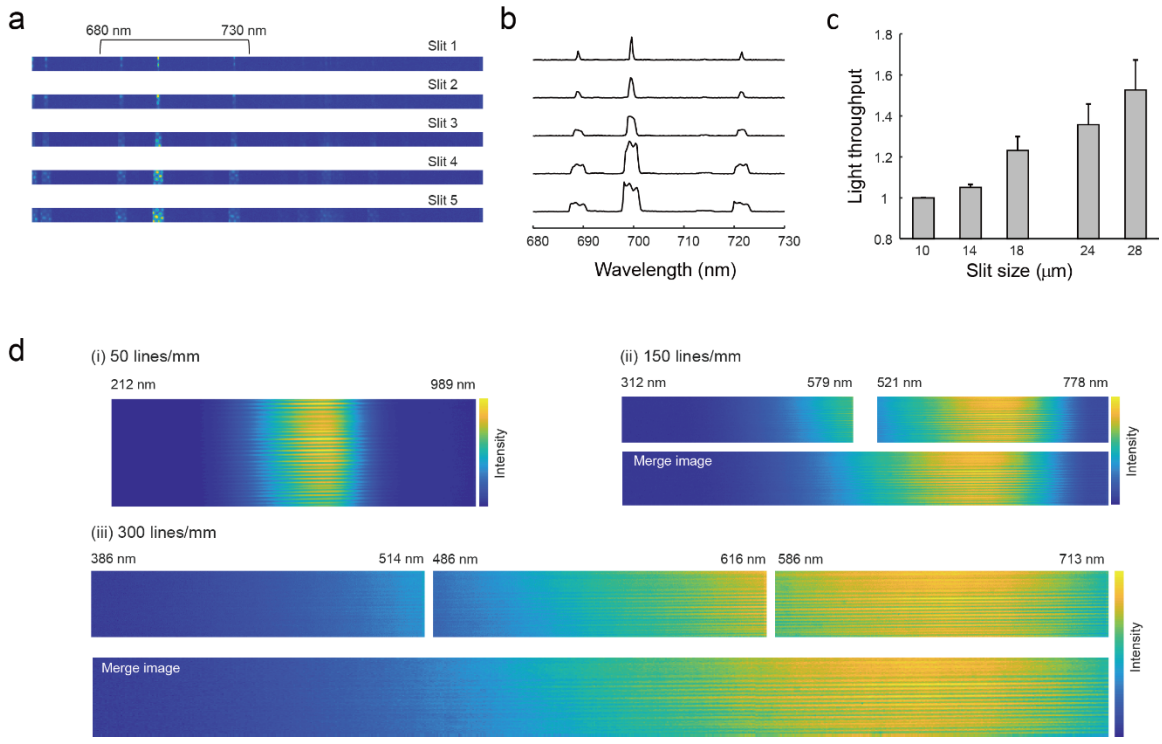


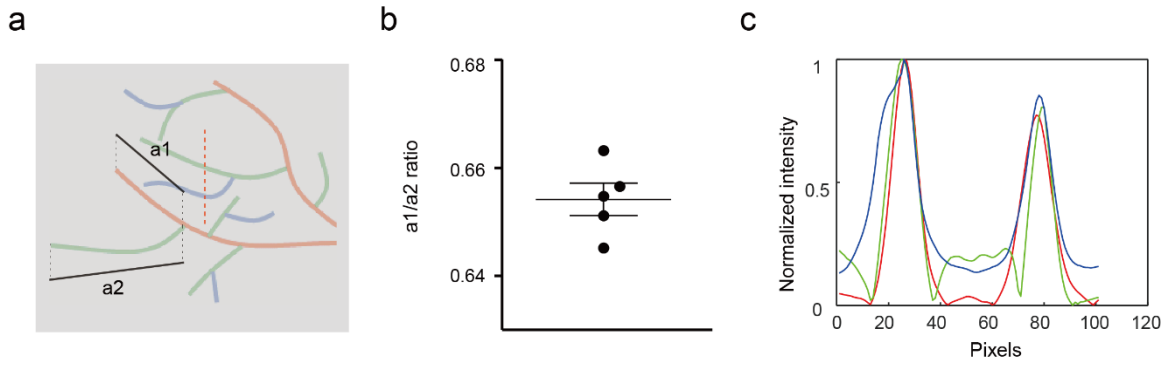

**Supplementary Figure. 1. A schematic overview of the workflow of hyperspectral endoscopic imaging. a** Hyperspectral and wide-field image measurement and normalisation. **b** Wide-field image registration process for estimating geometric transformation matrices (GMs) of each wide-field image to retrieve information about imaging position and distortion required for hypercube reconstruction. **c** Hypercube reconstruction process by exploiting normalised spectral images and estimated GMs.



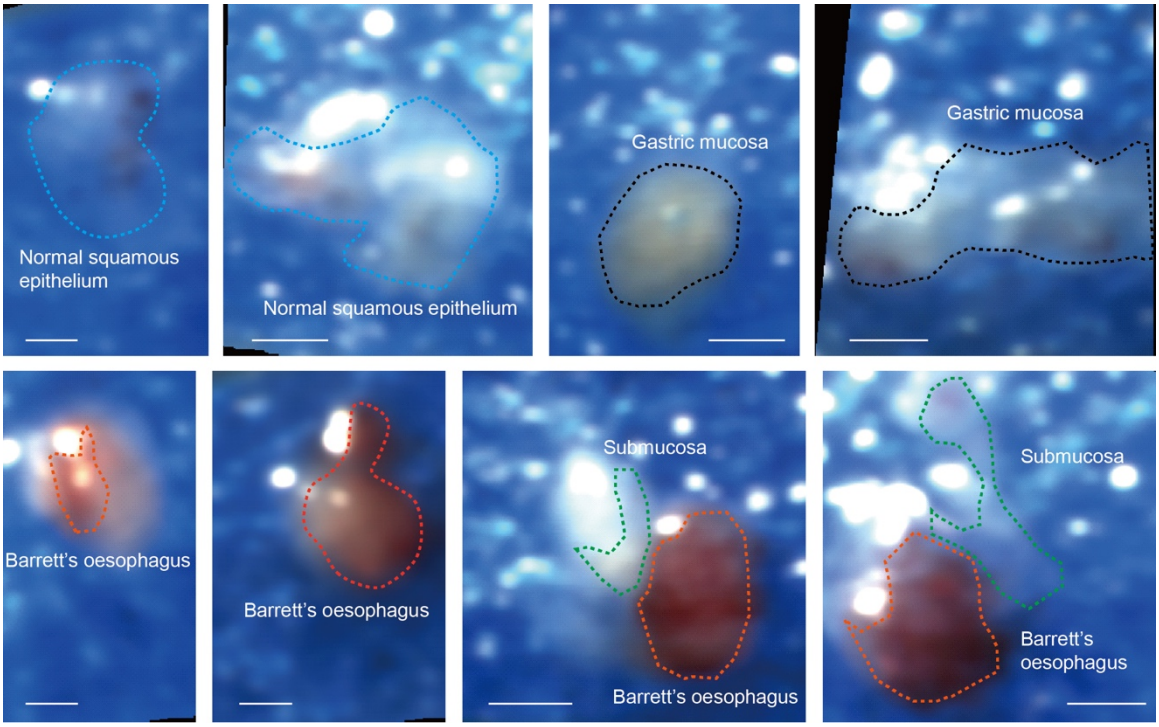

**Supplementary Figure. 2.** ***In silico*** **USAF 1951 test target used for simulation. a** 2D projection image of simulated hyperspectral USAF target along the wavelength. Scale bar = 200 pixels. **b** Six spectral features randomly assigned to each USAF target element.



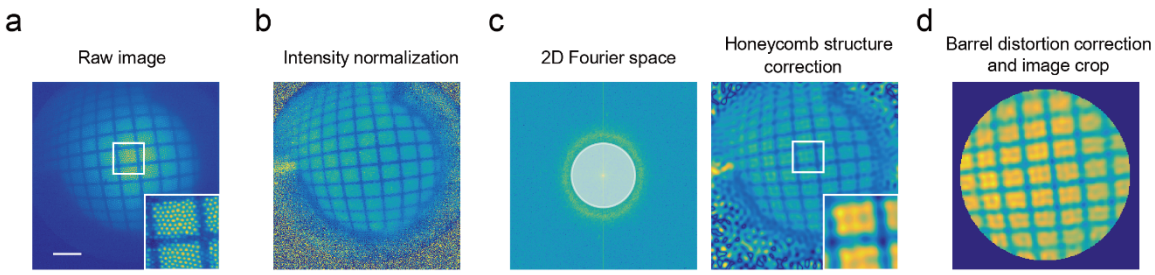

**Supplementary Figure. 3. Investigation of spectral resolution and bandwidth. a** Spectral images measured using five different slit sizes. **b** Line profiles of spectral images between spectral ranges of 680 to 830 nm. **c** Quantification of light throughput measured using five different slit sizes. Average light throughput measured using the 10 μm slit was used as a reference. Error bars represent standard deviation. **d** Spectral bandwidth of a single spectral images measured using three different gratings. To obtain a spectral image over 300 nm, multiple spectral images were merged.



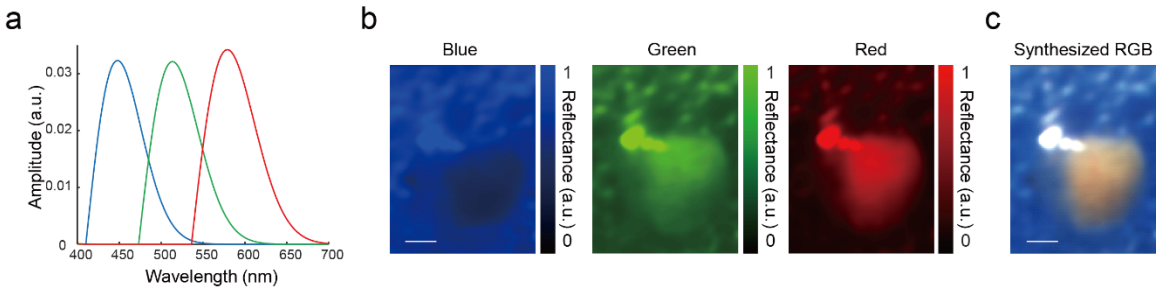

**Supplementary Figure. 4. Quantitative analysis of freehand imaging quality. a** A vascular tree phantom measured by free-hand hyperspectral imaging. **b** a1 and a2 ratio was quantified shown in a. Centre horizontal line indicates mean value and error bars represent standard deviation. **c** Line profile of the dashed red line shown in a.



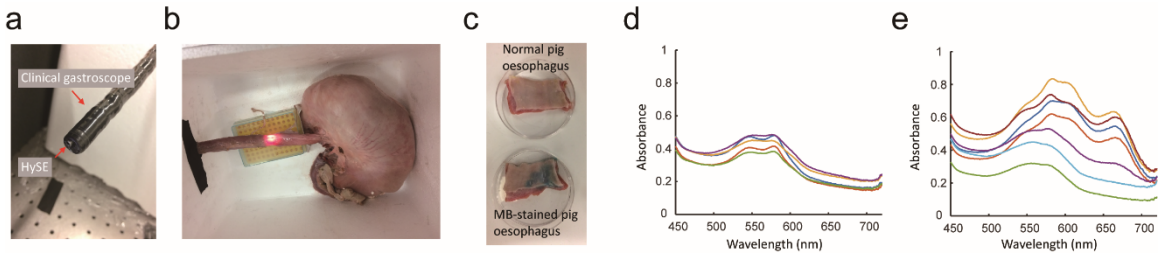

**Supplementary Figure. 5. Synthetic RGB images of *ex vivo* human tissues from patients.** Dashed line indicates boundary of each tissue type. Scale bars are 1 mm.




**Supplementary Figure. 6. Pre-processing steps for endoscopic image calibration. a** Raw endoscopic image. Inset shows magnified image of the white dashed square. Scale bar = 1 mm. **b** Intensity normalized image following correction with white and dark background images. **c** Honeycomb structure correction via low-pass filtering in 2D Fourier space. 2D Fourier transformed image (Left). Grey circle indicates the size of low-pass filtering mask. Image without honeycomb structure obtained by inverse Fourier transformation of filtered 2D Fourier information (Right). Inset shows magnified image of the white dashed square. **d** Image after all pre-processing, including barrel distortion correction and image crop.




**Supplementary Figure. 7. Generation of synthetic RGB image from the hyperspectral image.** **a** Spectral profile of applied red, green, and blue filters. **b** Blue, green, and red images of ex vivo human tissue generated by multiplying the corresponding colour filter in a by the reconstructed hypercube. **c** Synthesized RGB image of tissue. Scale bars = 1 mm.




**Supplementary Figure. 8. Experiment setup for hyperspectral imaging of an intact *ex vivo* pig oesophagus model. a** Photography of endoscopy setup. The HySE was inserted via an accessory channel of the clinical gastroscope. **b** Photography of the inflated pig oesophagus and stomach. Illumination at the distal end of the HySE using the internal illumination method can be seen as a bright area in the oesophagus. **c** Photography of the dissected pig oesophagus tissue with and without methylene-blue (MB) staining. Lumen of the pig oesophagus was exposed and measured using a spectrometer. **d, e** Ground-truth absorbance of normal and MB-stained pig oesophagus tissue was measured via the spectrometer at multiple random locations in the tissue.

Supplementary Table 1. Summary of specifications of HySE compared to nearest previous reports.

| | HySE | | | Previous works | |
|---|---|---|---|---|---|
| | 50 lines/mm | 150 lines/mm | 300 lines/mm | Ref 15 | Ref 54 |
| Operation mode | Spatial scanning | | | Rotating multicolour filter | Snapshot |
| Spectral resolution | ~ 2.85 ± 0.39 nm | ~ 1.10 ± 0.01 nm | ~ 0.46 ± 0.05 nm | ~ 15 nm | 4 to 10 nm |
| Bandwidth or spectral ranges | 750 nm | 250 nm | 125 nm | 400 – 760 nm | 450 – 650 nm |
| Spectral channels | Over 200 channels | | | 18 | 48 |
| Spatial resolution | 119.46 ± 11.58 μm @ working distance of 5 mm | | | N.A | 100 μm |
| Image acquisition speed | Without pixel binning: 20 fps (spectral imaging) | | | 15 fps | 8 to 10 fps |
| | 2 vertical pixels binning: 29 fps (spectral imaging) | | | | |
| | Over 35 fps (wide-field imaging) | | | | |