Technical Appendix

I. Detailed Parameters of Double Contrast-Enhanced Ultrasonography (DCEU)

1. Equipment and Probe Parameters

| Equipment Model | Probe Type | Application Scenario | Frequency Range | Scanning Mode | Imaging Depth |
|---|---|---|---|---|---|
| GE LOGIQ E9 (GE Healthcare, USA) | C1-6 Convex Abdominal Probe | Colon Tumor Scanning | 1 – 6 MHz (default: 3 MHz) | 2D Grayscale + Color Doppler + CEUS （contrast-enhanced ultrasound, CEUS ） Mode | 3 – 8 cm |
| GE LOGIQ E9 (GE Healthcare, USA) | IC5-9 Endorectal Probe | Rectal Tumor Scanning | 4 – 9 MHz (default: 6 MHz) | Endoluminal Grayscale + Color Doppler + CEUS （contrast-enhanced ultrasound） Mode | 2 – 5 cm |

2. Contrast Agent Parameters and Injection Protocol

| Contrast Agent Type | Manufacturer | Composition and Specification | Preparation Method | Injection Route | Injection Rate | Flushing Protocol |
|---|---|---|---|---|---|---|
| Intraluminal Gastrointestinal Ultrasound Contrast Agent | Huzhou Dongya Company | 50 g/package (containing Coicis Semen, Dioscoreae Rhizoma, Citri Reticulatae Pericarpium, etc.) | 50 g powder + 500 mL boiling water, stirred to dissolve, cooled to room temperature (20 – 25℃) | Transanal Perfusion | Adjusted according to patient tolerance (approximately 80 – 120 mL/min) | Scanning performed after intestinal lumen distension |
| Intravenous Ultrasound Contrast Agent (SonoVue) | Bracco SpA, Italy | Microbubble diameter: 2.5 μm, Concentration: 8 μL [45 μg]/mL | Used after reconstitution according to the product instructions: Inject 5 mL of normal saline into the vial of SonoVue lyophilized powder, gently invert to mix (avoid vigorous shaking). After reconstitution, it forms a milky white microbubble suspension, which is directly aspirated for use. | Peripheral Venous Injection | Injection rate ≈ 1 mL/s | 5 mL normal saline used to flush the catheter after injection |

3. Imaging Parameter Settings

| Imaging Mode | Gain Setting | Dynamic Range | Frame Rate | Scanning Duration | Data Storage Format | Key Parameter Notes |
|---|---|---|---|---|---|---|
| Intraluminal Perfusion Contrast Mode | 60 – 70 dB | 75 – 85 dB | 15 fps | Continuous scanning throughout the process | DICOM (static images) + MP4 (dynamic video clips) | Focal position fixed at the tumor center; no probe movement during scanning |
| Intravenous CEUS Mode | 55 – 65 dB | 80 – 90 dB | 30 fps | Arterial phase (10 – 30 s) focused collection; total 6 min | DICOM (static images) + MP4 (dynamic video clips) | "CEUS routine" mode adopted; no mid-scan parameter adjustment |

II. ROI Segmentation

1. Segmentation Personnel and Process

Segmenters: Two ultrasound radiomics physicians with 5 years (Physician A) and 10 years (Physician B) of clinical experience, both trained in ITK-SNAP software segmentation. The patients' pathological results (LVI status) were not disclosed to them before segmentation.

2. Segmentation Process

Physician A (with 5 years of clinical experience) used the open-source software ITK-SNAP to manually outline the Regions of Interest (ROI) frame by frame based on the images of the maximum tumor cross-section. Physician B (with 10 years of clinical experience) reviewed all ROIs manually segmented by Physician A to ensure the entire lesion area was fully included in the ROIs and normal tissues within the ROIs were excluded.

III. Supplementary Notes

All ultrasound equipment parameters were exported from the hospital's PACS system as original setting records;

ROI segmentation consistency verification data were calculated using Python 3.12.