Endometrium imaging using real-time rotational optical coherence tomography imaging system

A pilot, prospective and ex-vivo study

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Abstract

This study aimed to evaluate a novel real-time rotational optical coherence tomography (OCT) imaging system (OCTIS) with a fiber-optic probe to look at endometrium and to correlate the OCTIS images with standard histology. OCT could obtain real-time images resembling histological examination. With recent development of customized probes, it allows OCT to be used in the field of gynecology.

This is a pilot, prospective, ex-vivo and observational study. Women underwent hysterectomy for various gynecological conditions were recruited and OCTIS images were obtained from endometrium of 15 fresh uterus specimens immediately after hysterectomy. The excised uterus was cut open and OCTIS imaging was obtained. The scanned region of endometrium was excised for histological examination and OCTIS images were precisely compared to corresponding histological images and ultrasound images. Blinded qualitative analysis on OCTIS images was performed by 2 assessors to determine inter-rater reliability on the histopathological diagnosis.

Epithelium, glands, cysts, and stroma of endometrium were clearly seen by the OCTIS. Different phases of menstrual cycle of normal endometrium could be differentiated and pathological condition such as hyperplastic and dysplasic endometrium, which corresponded well with histological findings, could be identified. The inter-rater reliability between assessors on overall OCTIS endometrium and neoplastic OCTIS endometrium was moderate (Kendall $\tau_b$ of 0.58) and substantial (Kendall $\tau_b$ of 0.76), respectively.

OCTIS can provide real-time, high-resolution and rotational imaging modality to view endometrial structure with high consistency with histological examination and satisfactory agreement between observers. It has a great potential to be developed in the clinical use of endometrial assessment for gynecological applications.

Abbreviations: OCT = optical coherence tomography, OCTIS = optical coherence tomography imaging system.

Keywords: endometrium, gynecology, optical coherence, tomography, uterus

1. Introduction

Interventional and diagnostic imaging on endometrial tissue remains a challenge because of its deep location, and dynamic nature. Endometrial biopsies and histological examinations are necessary for final pathological diagnosis before definite and proper medical and surgical treatment, but it is invasive and time-consuming. An image modality that could provide instant, non- or minimal-invasive, high-resolution and reproducible examination of endometrium would; therefore, be of value to provide fast diagnosis for timely treatment.

Optical coherence tomography (OCT) was first applied to generate near-infrared optical images from surface tissues in early 1990s. It measures amount of backscattered signal at micrometer-scale resolution. The resolution of OCT is 100 to 250 times higher than that of ultrasound, approaching the resolution of microscopic histopathological examination. An image produced by OCT resembles the tissue architecture observed in histology and can; therefore, be considered as an “optical biopsy.” Earlier OCT system used handheld devices for imaging, thus its clinical applications are limited to surface organs such as skin and retina. There are few groups that attempted to use OCT in female reproductive organs but their focus is mainly on cervix, fallopian tube, vagina, and recently on ovary. Kirillin et al demonstrated accuracy of OCT in...
detecting fallopian tube with pelvic inflammatory diseases as high as 88%.[16] In 1999[17] OCT was used on endometrium to detect endometrial neoplasms. It shows potential use of OCT to discern different endometrial pathology, such as endometrial carcinoma and different phases of endometrium, but the image quality was still not optimal and the correlation of OCT images with histology on different morphology of endometrium is also lacking.

One of the reasons that the conventional OCT is not widely used in endometrium as the current OCT device are mostly facilitated with hand-hold probe; longitudinal optical imaging for ductal organs was not readily accessible.[12] Newer generation OCTIS had been developed to incorporate catheter-based, fiber-optic, rotational probes with improved OCT technologies which can be adapted for ductal organ imaging with and without endoscopy, such as coronary OCT.[18]

Using OCT in combination with fiber-optic may enable the imaging of lining of internal organs, such as endometrium. The OCTIS can obtain non- or minimally-invasive, real-time, high-resolution, cross-sectional, rotational positioning and quantitative images of epithelial or sub-epithelial tissues in a ductal organ.[12] The objective of this study was to evaluate the use of OCTIS on endometrium of various pathologies ex-vivo and to establish any correlation between OCT images and standard histology. Since reliability determines the value of a diagnostic tool, inter-rater reliability was also tested in this study.

2. Methods

2.1. Ethical issue

This is a pilot prospective observational study approved by the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee (Clinical Research Ethics: CRE-2016.160). Written informed consent was obtained before hysterectomy (Supplementary document S1).

2.2. Subjects

A total of 15 women were recruited in this study. These women underwent hysterectomy for various gynecological conditions. Inclusion criteria included women of any age, regardless of menstrual cycle and status, with indication for abdominal hysterectomy. Exclusion criteria included women underwent laparoscopic hysterectomy with potential disruption to endometrium during hysterectomy.

2.3. Optical coherence tomography imaging system

OCTIS (Tomophase Inc, Cambridge, MA; and Guangdong Winstar Medical Technology Co, China) is a new frequency domain based OCT system (Fig. 1). It consists of an imaging computing system (Fig. 1A) and a sterile detachable probe (Fig. 1B). The imaging computer system has a designated program to display, save and report real-time OCT images. The rotating fiber-optic probe is a catheter measures 2.5mm in diameter and 15cm in length which is sealed with a transparent outer sheath with a 1mm long window near its tip for image scanning and capturing. Side-viewing probe was chosen as it is suitable for the imaging of tube-like hollow organs such as uterine cavity. There is a flexible fiber optical shaft for rotational imaging at 600 to 1200rpm inside the sheath. The optical fiber is illuminated by a broad wavelength-range light source operating at 1300nm and a sweeping rate of 50kHz. The images acquisition speed is at 20 frames per seconds with 15 μm axial and 25 μm lateral resolution and depth up to 3 mm in both grey and color modes. The scanning window at the distal tip of the catheter is brought to the area of interest to capture OCT image. The OCTIS is FDA (K102599) approved for medical imaging; this machine has been evaluated in respiratory medicine to study nasal mucosa of cystic fibrosis.[19]

This study was conducted in a tertiary hospital. Demographic information was obtained from all recruited women, followed by physical examination and pelvic ultrasound examination before the operation. All women underwent total abdominal hysterectomy with or without other relevant procedures according to the nature of disease. After hysterectomy, the excised uterus was collected and cut opened immediately to expose the whole endometrium. The endometrium imaging was carried out using OCTIS probe placed on the endometrium from fundal region of the uterus to endocervical canal continuously. Images were captured and saved. The endometrial tissue at corresponding OCTIS scanned region was excised for standard pathological examination by experienced pathologist.

2.4. Evaluation of histopathology versus OCTIS images

Each collected endometrium was first marked by hematoxylin on the endometrial surface to ensure subsequent histological analysis was performed on the same region of interest of the endometrium OCTIS imaging. Afterward, the endometrium was fixated in 10% neutral formalin immediately, and then processed with serial ethanol dehydration and paraffin-embedded procedures by pathologist. Endometrial sections of 4 μm thickness were cut by microtome, and slides were stained by hematoxylin and eosin. Tile scan images were acquired by Leica microscopy. The histopathologic features such as phase of endometrium, endometrial polyp, endometrial hyperplasia, and other pathological features were correlated with OCTIS images in the aspect of morphology, thickness, tissue structure, and distribution. Ultrasound images performed before hysterectomy were also used to compare with OCTIS images.

2.5. Qualitative data analysis

To assess the reliability of OCT images on endometrium, qualitative data analysis was conducted. After histopathological evaluation, the corresponding OCTIS images on the same region of interest for each of the histological categories (categorical variables); namely “proliferative endometrium,” “secretory endometrium,” “inactive endometrium,” “cystic endometrium,” “endometrial hyperplasia with atypia,” and “endometrial carcinoma” were saved to a database. Subsequently, all OCTIS images were selected and mixed; and handed over to 2 assessors (TSML and FRW) blinded to the clinical information and histological results for independent analysis.

2.5.1. Sample size. The quantitative analysis aimed to determine the inter-rater reliability for the pathology on endometrium under 6 categories as stated above. The minimum value for the correlation coefficient to be expected by the assessors was 0.4 when no agreement among the assessors was assumed at the first place. When the power and alpha were specified at 80% and 0.05, respectively, a minimum sample of 13 subjects was required to detect a minimum value of correlation coefficient of 0.4.[20]
2.6. Statistics

Data were analyzed using the Statistical Package for the Social Sciences (Windows version 22.0; SPSS Inc, Chicago, IL). Inter-rater reliability was calculated for each histopathological diagnosis and expressed in the Kendall $\tau_b$ correlation coefficient. Coefficients were interpreted according to Landis and Koch\textsuperscript{[21]} as “poor” ($<0.0$), “slight” ($0.0–0.20$), “fair” ($0.21–0.40$), “moderate” ($0.41–0.60$), “substantial” ($0.61–0.80$), and “excellent” ($0.81–1.00$). $P$-value of $<.05$ was regarded as statistical significant.

2.7. Data availability

All data were recorded into a structured datasheet and all OCTIS and pathohistological image were stored in a database. Although the data was not deposited in a publicly available resource, all authors had full access to all the data (including all images, statistical reports, and tables) in the study and can take responsibility for the integrity of the data and accuracy of the data analysis.

3. Results

A total of 15 women with different indications for hysterectomy were included in the study. The age of the women ranged from 40 to 84 years. The indications of hysterectomy included uterine fibroid, adenomyosis, atypical complex hyperplasia of endometrium, endometrial cancer, and ovarian cancer. Summary of demographic characteristics and clinical and pathological diagnosis of recruited women is presented in Table 1. A total of 145 OCTIS images were captured on the 15 uterine specimens, on average 9 to 15 images per specimens.

3.1. Histopathology vs OCTIS images

There were a total of 150 corresponding slides, some were damaged during sample collection and could not be used; consequently, a total of 125 usable slides were used in this study. Matching of OCTIS images to the corresponding histology was performed. Based on the classification of histopathology, representative OCTIS images and histological pictures of proliferative endometrium, secretory endometrium, inactive endometrium, cystic endometrium, endometrial hyperplasia with atypia, and endometrial carcinoma are shown in Figures 2–7.

For different phases of endometrium, histopathological and OCTIS images were well matched with unique characteristics. For proliferative endometrium (Fig. 2), OCTIS could distinguish functional endometrial layer and myometrium; therefore, endometrial thickness could be precisely measured. However, luminal epithelium could not be easily distinguished from the endometrium on the OCTIS. Endometrial glands could also be seen clearly particularly on the colored OCTIS images. For ultrasound, the endometrium appears thin with thickness in 2 mm.

For mid-secretory phase endometrium (Fig. 3), the endometrial thickness is beyond 3 mm, which is beyond the maximum depth
of OCTIS. There are multiple dilated glands with regular tortuosity identified which were shown as low contrast pattern in a background of bright signal field. The corresponding ultrasound image showed a thickened homogenous endometrium with thickness in 12 mm. For inactive endometrium (Fig. 4), the basal line could be easily distinguished especially on the black and white OCT with brighter signal and higher density being the endometrium and lower signal being the myometrium. The glands seen were sparse and small (<1 mm). The corresponding ultrasound findings showed a thin midline, and the endometrial echo on ultrasound was suboptimal as rendered by artifact of uterine fibroid. Cystic atrophic endometrium (Fig. 5) is characterized by multiple low signal cystic spaces with size 0.5 to 1 mm. There is intense signal in between the cystic spaces corresponding to atrophic stroma. The OCTIS images demonstrated high correspondence to histology. For ultrasound, the endometrium thickness was about 3 mm and hardly showed any cystic characteristics.

For endometrial hyperplasia with atypia (Fig. 6), it is more difficult to differentiate from normal endometrium. With histology in low magnification (Fig. 6A), there were marked crowded glands of various sizes with increased gland/stroma ratio. The variably crowded glands could also be observed in OCTIS images (Fig. 6B and C) as homogenous tissue structure. Atypical cells would require much higher power field and could not be identified on OCTIS. With the presence of endometrial polyp, ultrasound could not measure endometrial thickness accurately and it could not show any clue for endometrial hyperplasia.

Figure 7 shows a case of endometrial carcinoma. Defined organization of epithelial layers and glands was no longer present. The endometrium was shown as a layer of high-density heterogeneous signal on OCTIS images likely due to high cell density.

![Figure 2](image-url)

**Figure 2.** Proliferative phase endometrium demonstrated by parallel histology (A) and OCTIS images (B and C) and transvaginal ultrasound (D). Luminal epithelium (red arrows), functional endometrial thickness (area between blue arrows), myometrium (area below green arrow) and endometrial glands (yellow arrows) could be observed on OCTIS images. OCTIS = optical coherence tomography imaging system.
density. The OCTIS images corresponded to the histology appearance with elongated and slit-like glandular space lined by columnar epithelial cells. In ultrasound the endometrial tumor appeared as hyperechoic irregular mass in endometrial cavity.

Table 2 summarized the comparison of the performance of ultrasound, OCTIS (black and white image) and OCTIS (colored image) on endometrium in different pathology. OCTIS can identify lumina epithelium in most of the cases except for the endometrial carcinoma case. However, it could not visualize the cellular structures to aid further detailed endometrium lining examination. Endometrial glands are well visualized on OCTIS images as low signal area in all cases except high-density heterogeneous signal in endometrial carcinoma. For myometrium, since its distance from endometrium is further, the resolution and assessment of myometrium by OCTIS are limited. Colored OCTIS images appear to be superior to black and white...
images as it could better demonstrate the intensity of signal and thus the density of tissue. Colored OCTIS could show stroma better than black and white OCTIS. Overall, OCTIS is superior to ultrasound in assessing endometrial pathology.

3.2. Qualitative data analysis

Two assessors performed independent blinded assessment on the OCTIS images for the histopathological diagnosis. The results are shown in Table 3. The percentage on the table shows the number
of OCTIS images classified correctly by the assessors. The concordance for cystic endometrium was 100% for both assessors. As regards proliferative, secretory, and inactive endometrium, the concordance ranged from 69.2% to 93.7%. Endometrial hyperplasia with atypia has the lowest concordance rating (40% by assessor A and 53.3% by assessor B). The sensitivity and specificity of malignancy detection is 76.9% and 86.6% for Assessor A and 84.6% and 72.2% for Assessor B. The inter-rater reliability on overall OCTIS images was calculated and resulted in a Kendall $\tau_b$ of 0.58, meaning moderate agreement between assessors. Most of the histological pathologies resulted in substantial to excellent inter-observer reliability (Kendall $\tau_b$ ranges from 0.488 to 1.00) (Table 2). On the other hand, the inter-rater reliability on OCTIS images for malignancy has a substantial agreement (Kendall $\tau_b$ of 0.76) between the 2 assessors.

4. Discussion
This study demonstrated high correspondence of OCTIS images of endometrium to different histology and could even distinguish benign to malignant endometrium. Based on our result,

![Figure 7](image)

Figure 7. Endometrial carcinoma: endometrial carcinoma demonstrated by parallel histology (A), OCTIS images (B and C) and transvaginal ultrasound (D). Luminal epithelium (red arrows), multiple irregular dilated glands (yellow arrows) could be clearly seen in histology and OCTIS images. The endometrial thickness (blue arrows) could be measured on ultrasound. OCTIS = optical coherence tomography imaging system.

| Pathology                        | Luminal epithelium | Functional endometrium | Endometrium glands | Stroma | Myometrium | Micro-vessels | Pathology |
|----------------------------------|--------------------|------------------------|--------------------|--------|------------|--------------|-----------|
| Normal proliferative phase       | USG                | +                      | --                 | --     | ++         | --           | NA        |
|                                  | OCTIS bw           | +                      | ++                 | ++     | --         | --           | NA        |
|                                  | OCTIS color        |                        | ++                 | ++     | --         | --           | NA        |
| Mid-secretary phase              | USG                | --                     | --                 | --     | ++         | --           | NA        |
|                                  | OCTIS bw           | --                     | ++                 | ++     | --         | --           | NA        |
|                                  | OCTIS color        | --                     | ++                 | ++     | --         | --           | NA        |
| Fibroid, inactive endometrium    | USG                | --                     | --                 | --     | +          | --           | ++        |
|                                  | OCTIS bw           | --                     | ++                 | ++     | --         | --           | +         |
|                                  | OCTIS color        | --                     | ++                 | ++     | --         | --           | +         |
| Cystic atrophic endometrium      | USG                | --                     | --                 | --     | +          | --           | --        |
|                                  | OCTIS bw           | --                     | ++                 | ++     | --         | --           | +         |
|                                  | OCTIS color        | --                     | ++                 | ++     | --         | --           | ++        |
| Endometrial hyperplasia          | USG                | --                     | --                 | --     | +          | --           | --        |
|                                  | OCTIS bw           | --                     | ++                 | ++     | --         | --           | +         |
|                                  | OCTIS color        | --                     | ++                 | ++     | --         | --           | +         |
| Endometrioid carcinoma           | USG                | --                     | --                 | --     | +          | --           | --        |
|                                  | OCTIS bw           | --                     | ++                 | ++     | --         | --           | +         |
|                                  | OCTIS color        | --                     | ++                 | ++     | --         | --           | +         |

NA = not applicable, OCTIS = optical coherence tomography imaging system, OCTIS bw = OCTIS in black and white, OCTIS color = OCTIS in color, -- = no better than others, + = slightly better than others, ++ = better than others, +++ = much better than others.
endometrial epithelium, glands, and stromal tissues could be observed on OCTIS. These conditions are usually not easily detected or differentiated on ultrasound or direct visualization using hysteroscopy. From our result, substantial to excellent correlation between the 2 assessors was achieved in assessing secretory endometrium, inactive endometrium, cystic endometrium, and endometrial carcinoma respectively due to the specific characteristics on OCTIS images such as the cystic appearance in cystic endometrium; distinctive dilated gland in secretory endometrium; sparse and small glands in inactive endometrium; and high density signal in endometrial carcinoma due to high cell density. On the other hand, moderate correlation between the 2 assessors was obtained for proliferative endometrium and endometrial hyperplasia with atypia. It is likely due to lack of strong characteristics in proliferative endometrium when compared to other phases of endometrium; and it is more difficult to differentiate hyperplasia from other pathologies as atypical glands could only be viewed on microscopy. The satisfactory correlation between the 2 assessors demonstrated it is possible to use OCTIS to differentiate different pathologies with training. Ideal training would include introducing the characteristics of each tissue pathology and the matching of characteristics on OCTIS images; followed by test on multiple images. The encouraging findings fulfilled the objective of this study as the first step of using OCTIS on endometrium so we could perform further in-vivo and clinical studies base on our findings. The high-resolution OCTIS image of endometrial morphology may add valuable information to guide clinical management timely without waiting for a histological diagnosis.

In the study on detecting endometrial neoplasm with conventional OCT, the OCT image could not delineate different layers of endometrium and could only give a rough idea of the possible pathology. Our study using an improved OCT technology illustrates the potential use of real-time rotational OCT on endometrium with its good correlation to histology, and demonstrate the ability of the new OCTIS in differentiating different layers of endometrium such as luminal endometrium, functional endometrium, and endometrial glands which are currently not accessible by ultrasound and hysteroscopy. Ultrasound is widely used to assess endometrium and there are different features for different phases of endometrium. However, different phases of endometrium are mainly differentiated by endometrial thickness while this study shows OCTIS could demonstrate endometrial glands which could add information on the assessment of different phases of endometrium. In addition, the OCTIS machine used in our study can obtain real-time images without delay as it took 10 to 30 seconds for the endometrium evaluation in the above study that render clinical use of OCTIS. OCTIS could examine endometrium in a minimally invasive way and is more superior to ultrasound as per the findings of our study.

One of the drawbacks of using the OCTIS on endometrium is its limitation on the depth of image. The single-layer endometrium could be as thick as 5 to 7 mm in secretory phase while the maximum depth detected by the OCT is 3 mm in current technology. Nevertheless, OCTIS has an important advantage over standard histopathology because this new OCTIS can be applied in-vivo, non- or minimally-invasive and there is great potential to study on the pathology on superficial endometrium. With recent advance of OCT technology in a considerable pace, improvement in resolution and depth of the OCTIS machine would not be far and shall overcome the image depth issue. Another drawback is interpretation of OCT images which is operator dependent and one requires experience with this new imaging modality as in the beginning of ultrasound imaging. Future trends of development could aim to develop automation of image assessment to unify the analysis.

There are some limitations of the present study. First, for the study design, it is an ex-vivo prospective study. The application of OCTIS in-vivo is required and its accuracy and safety need to be further evaluated. Second, the OCTIS has not been compared to hysteroscopic findings, which are commonly used modalities to assess endometrium in current clinical practice. Third, the OCTIS images of the same region were assumed to be the same histopathology while it is possible that some areas may contain focal tissue of a different pathology such as hyperplasia. Fourth, in regard to qualitative assessment, since literature data on OCT images of the endometrium is limited, the experience of the assessors is at the learning phase; with more samples available and further training on viewing the OCTIS images with correlation of histopathology, better concordance results are expected. The sensitivity of endometrial carcinoma detection by visual assessment reported in our study is 76.9% and 84.6%, respectively by the 2 assessors, with the limitation that the 15 OCTIS images all came from 1 subject only; it is possible that OCT images may vary in other patients with endometrial carcinoma. Finally, in this pilot study, there was no quantitative analysis of the OCT images for analysis and interpretations especially in the comparison with ultrasound images. To confirm the clinical usefulness of the OCT, further in vivo, comparison and quantitative studies are in progress in our center.

### Table 3

| Histopathology                          | Assessor A | Percentage (%) | Inter-rater reliability | Inter-rater reliability |
|-----------------------------------------|------------|----------------|-------------------------|-------------------------|
|                                        | Correct/number of OCT image | | Kendall \( r_b \) (p-value) |         |
| Proliferative endometrium               | 53/65      | 81.5%          | 45/65                   | 69.2%                   | 0.488 (\( P = .01 \)) Moderate |
| Secretory endometrium                   | 8/10       | 80%            | 9/10                    | 90%                     | 0.652 (\( P = .026 \)) Substantial |
| Inactive endometrium                    | 28/32      | 87.5%          | 30/32                   | 93.7%                   | 0.681 (\( P = .000 \)) Substantial |
| Cystic endometrium                      | 10/10      | 100%           | 10/10                   | 100%                    | 1.000 (\( P = .000 \)) Excellent |
| Endometrial hyperplasia with atypia     | 6/15       | 40%            | 8/15                    | 53.3%                   | 0.531 (\( P = .023 \)) Moderate |
| Endometrial carcinoma                   | 10/13      | 76.9%          | 11/13                   | 84.6%                   | 0.760 (\( P = .017 \)) Substantial |

OCT = optical coherence tomography.
5. Conclusions
To conclude, the result of this pilot study showed that OCTIS can provide real-time, high-resolution and rotational imaging modality to view endometrial structure with high consistency with histological examination and satisfactory agreement between observers. These findings open a new potential imaging approach to examine the endometrial structures and could serve as basis for future in vivo study. Further studies to delineate the clinical applications of the OCTIS in the imaging of the endometrium in various clinical conditions seem to be justified.

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