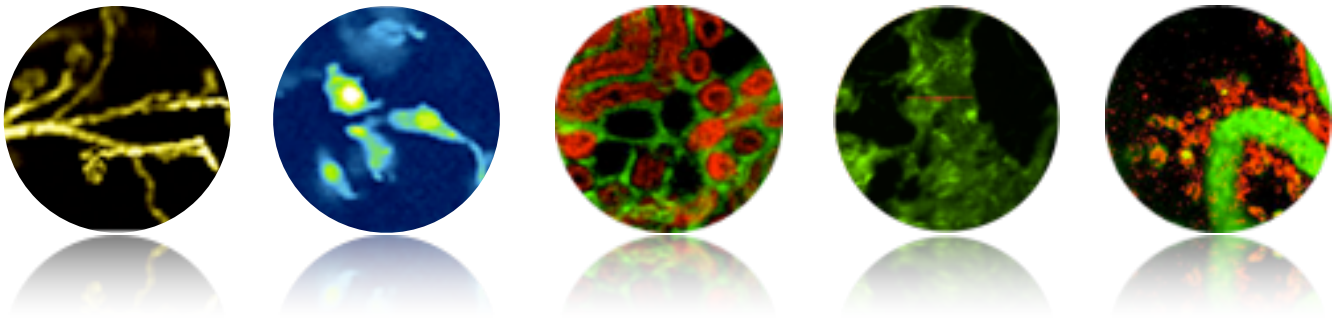
Post-International Conference of Cellvizio Users (ICCU) report

Confocal Laser Endomicroscopy Image interpretation: an update



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Introduction: Criteria for Image Interpretation, an Update.

This document follows on the 2013 edition of the Post-International Conference of Cellvizio Users(ICCU) report. With more than 200 peer-reviewed clinical publications, this updated version is intended to provide the most recent clinical results in regard to the current indications of confocal laser endomicroscopy in gastroenterology, pulmonology and urology. This includes results of multicentric randomized trial in gastroenterology luminal indications, as well as groundbreaking results in pancreatic indications, now accessible «through» the needle. The latest developments achieved in pulmonary & urology indications are largely detailed, providing further details on the use of CLE in those indications, as well as a comprehensive atlas of images.

A first state-of-the-art classification system for normal and pathological states in gastrointestinal diseases using probe-based Confocal Laser Endomicroscopy (pCLE) was established in 2009 in Miami by a group of experts during the first ICCU: the Miami classification (1). As pCLE was quite new at that time, few individuals had significant expertise in each field. Therefore, the standards were mainly based on expert opinion, and consensus development. Moreover, the biliary exploration with endomicroscopy had just started, and experience in using pCLE for indeterminate strictures was limited. Since then, the following method for refinement of criteria was used:

- acquisition and review of unblinded CLE sequences with corresponding pathological confirmation
- description of morphological features, identified in the CLE images, and correlation with specific tissue diagnoses
- prospective in vivo validation of the proposed classification in cases blinded to histology and evaluation of diagnostic performances of the technique together with interobserver agreement.

Major clinical trials with larger sample size have been conducted. They aimed at assessing the diagnostic efficacy of pCLE with the Miami classification criteria, and refining these criteria to improve its efficacy (2), (3). Smaller studies have also been conducted by physicians, who wanted to research a specific disease or condition in greater detail (4,5). Besides, a new digestive procedure enabling observation of pancreatic cysts was implemented, called the needle-based Confocal Laser Endomicroscopy (nCLE). Further developments in various indications, such as inflammatory bowel diseases (IBD), stomach lesions, and colorectal polyps have been conducted. Finally, the urology and pulmonology indications, which are currently studied to expand the range of CLE applications, are presented.

Methodology for Criteria Definition

There is no obvious histopathology correlation between CLE images and ex vivo samples sent for pathological analysis. Indeed, CLE offers a dynamic and longitudinal microscopic view of the tissue, whereas tissue sampling offers a static and transverse view of the tissue (Figure 1). In luminal indications, a comparison between CLE biopsy and histology is possible, when identifying glands, crypts or goblet cells for instance. In ductal indications and solid organs however, such a parallel is not possible. However, speculation can be made to interpret the criteria defined : white bands with flow reflect blood vessels and increased neovascularization as is often found in neoplasia ; black bands with flow might resemble lymphatic vessels ; clumps and epithelium structures most likely correspond to tissue proliferation of malignant cells.

WHAT IS OPTICAL BIOPSY?

Current main indications for pCLE in digestive endoscopy include Barrett's esophagus (BE) surveillance and treatment, surveillance and treatment of gastric lesions, post-resection follow-up of colorectal lesions, assessment of inflammation and neoplasia in inflammatory bowel diseases, diagnosis of indeterminate biliary strictures and characterization of pancreatic lesions. This field of indications has extending. Technical developments including miniaturization of probes will offer new opportunities to extend the field of indications to other organs. The following table lists these potential applications in order of the current level of clinical evidence.

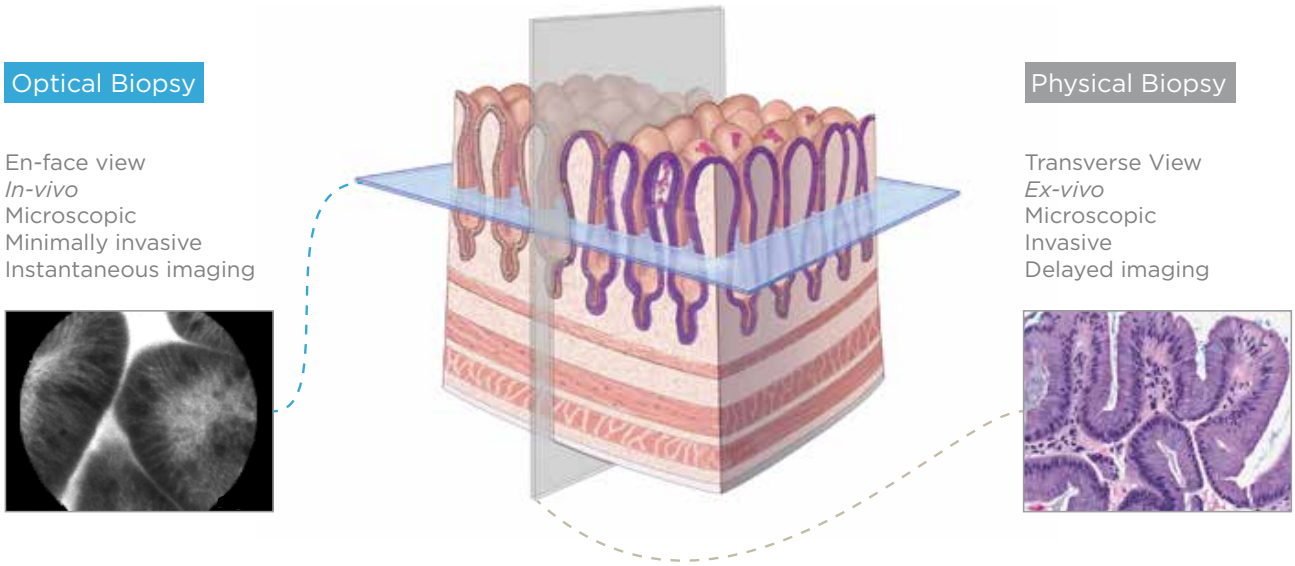

Fig.1: Comparison of optical biopsy versus physical biopsy

Cellvizio Indications

Established clinical validation	Ongoing clinical validation	Experimental areas
• Surveillance and treatment of Barrett's Esophagus	• Characterization of pancreatic lesions (pancreatic strictures, pancreatic masses)	• Surgery (digestive and abdominal)
• Detection of biliary cancers	• Primary sclerosing cholangitis	• Biomarkers
• Follow-up of colorectal endoscopic mucosal resection (EMR)	• Characterization of lymph nodes	• Gynecology
•Colorectal polyp differentiation	• Treatment and monitoring of inflammatory bowel diseases	• Neurosurgery
•Characterization of pancreatic cysts	• Characterization of pulmonary lesions	• Ear, Nose and Throat
• Characterization of gastric lesions	• Detection and treatment of bladder, kidney, prostate cancer	

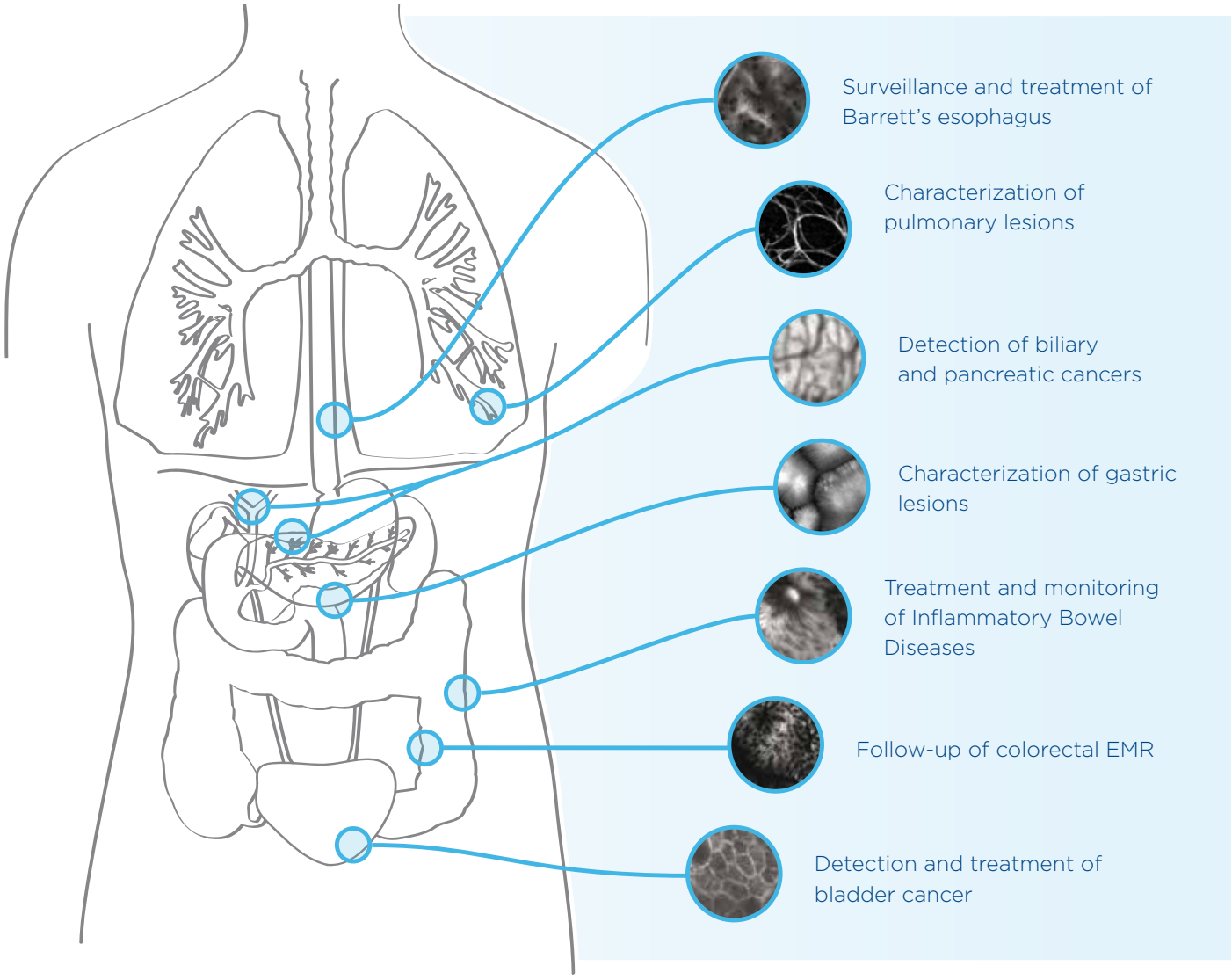

Fig.2: Cellvizio for a various clinical specialities

1. Digestive Luminal Indications

1.1. Barrett's Esophagus

Current guidelines for the surveillance of patients with BE recommend the Seattle protocol, which consists of taking 4-quadrant biopsies every 1 cm down the BE segment. However, physicians do not often adhere to this protocol, because it is time-consuming, cumbersome, and prone to sampling error (6). Areas with (pre)malignant lesions such as low-grade dysplasia (LGD), high-grade dysplasia (HGD) and cancer, are focal, and can be missed by physical biopsies. To achieve proper disease identification, advanced endoscopic techniques such as CLE have been developed to improve the detection of dysplasia in BE patients. Classification of CLE images as non-dysplastic versus dysplastic has been evaluated in several studies, from a pilot study to randomized multicentric trials, using evolving criteria (7,8,9). The Miami classification has been tested and validated in a large, multicentric randomized controlled trial, the DONT BIOPCE trial. Independent blinded endoscopists performed tandem endoscopic procedures to evaluate prospectively the sensitivity and specificity of pCLE in addition to white light endoscopy for the detection of HGD and early carcinoma (EC) in BE. The final results were published in 2011 (7). 101 BE patients presenting for surveillance or endoscopic treatment of HGD/EC were examined by high-definition white light endoscopy (HD-WLE), narrow-band imaging (NBI), and pCLE, and the findings were recorded before biopsy samples (874 samples) were obtained. On a per-location basis, the combination of pCLE with HD-WLE was twice as sensitive as HDWLE alone (68,3% for pCLE or HD-WLE versus 34,2% for HD-WLE alone). The addition of pCLE to HD-WLE or NBI was also more sensitive (1,7 times more) than HDWLE or NBI alone (75,8% for pCLE or HDWLE or NBI versus 45% for HD-WLE or NBI). Use of pCLE in conjunction with HDWLE and NBI thus resulted in detection of all HGD/EC patients (100% sensitivity). On a per-patient basis, the combination of pCLE with HDWLE or NBI achieved a sensitivity and NPV of 100% each.

This improved sensitivity for the detection of additional neoplastic areas could have a significant impact on the clinical management in the era of endoscopic treatment of HGD/EC through negative biopsy elimination, focal treatment guidance and residual neoplasia detection during follow-up. Following this prospective trial, a retrospective study was conducted by Gaddam et al. (10) in order to refine the pCLE criteria classification for dysplastic BE (phase 1), and to evaluate accuracy, inter-observer variability, and learning curve in dysplasia prediction (phase 2). Of multiple pCLE criteria tested (phase 1, using 50 pCLE videos), only those with >70% sensitivity or specificity were included in the final set: saw-toothed epithelial surface, enlarged cells, pleomorphic cells, non-equidistant glands, glands unequal in size and shape, and goblet cells not easily identified (Figure 2). Overall accuracy of pCLE in diagnosing dysplasia was 81.5%, and inter-observer agreement (IOA) was substantial (kappa=0.61). There was no difference between experts versus non-experts, suggesting a short learning curve. When the endoscopist was confident in making a diagnosis, accuracy rates were significantly higher (98% versus 62%). This makes a case that an endoscopist may only need to biopsy the areas he is not confident about.

The American Society for Gastrointestinal Endoscopy (ASGE) recently published a document entitled PIVI (Preservation and Incorporation of Valuable endoscopic Innovations), to provide guidelines for the adoption of virtual biopsy techniques (11). The thresholds that have to be achieved by the endoscopic innovations are 90% sensitivity, 80% specificity and 98% NPV for detecting HGD or early esophageal adenocarcinoma on a per-patient basis. The results obtained in the DONT BIOPCE trial were 100% sensitivity, 56% specificity, and 100% NPV on a per-patient basis. Therefore, pCLE could be considered for this initiative, given the potential of pCLE in the diagnosis of lesions in BE patients.

Johnson et al (12) report the use of pCLE in combination with WLE on the margins of previous resections to examine areas of intestinal metaplasia, dysplasia or cancer. This

study showed that pCLE enables endoscopists to identify dysplasia in real time, to target dysplastic areas for therapeutic treatment, to locate residual post-therapeutic areas for re-treatment, and then to confirm that ablated/resected areas and margins are free of residual intestinal metaplasia or dysplasia.

Bertani et al (8) enrolled 100 patients in a BE surveillance program, assigned by randomization to undergo HD-WLE only (50 patients) only or pCLE in addition to HDWLE evaluation (50 patients). The results showed that incidental dysplasia can be more frequently detected by pCLE than by HDWLE in BE. The higher dysplasia detection rate provided by pCLE could improve the efficacy of BE surveillance programs. Additionally, Sharma et al (13) have recently proved that pathologists appear to have similar accuracy and IOA as endoscopists. These results provide further support of endoscopists accurately interpreting the in vivo optical histology provided by pCLE. The impact of the CLE technique on the management of patients with BE has recently been demonstrated in a randomized multi-centric controlled trial by Canto et al (9). The study aimed at comparing HDWLE alone with random biopsy (RB) and HDWLE + eCLE and targeted biopsy (TB) for diagnosis of BE neoplasia on a large cohort of 192 patients. The corresponding results show that the association of CLE and HDWLE triples the diagnostic yield of physical biopsies and increases the sensitivity for neoplasia detection to 96% (compared to 40% for the standard approach). These performances could have resulted in a reduction in the number of physical biopsies by 80% as well as an improvement in the treatment plan in 36% of the patients. CLE can significantly impact *in vivo* decision making by altering the diagnosis and guiding therapy and has the potential to meet the thresholds set by the ASGE.

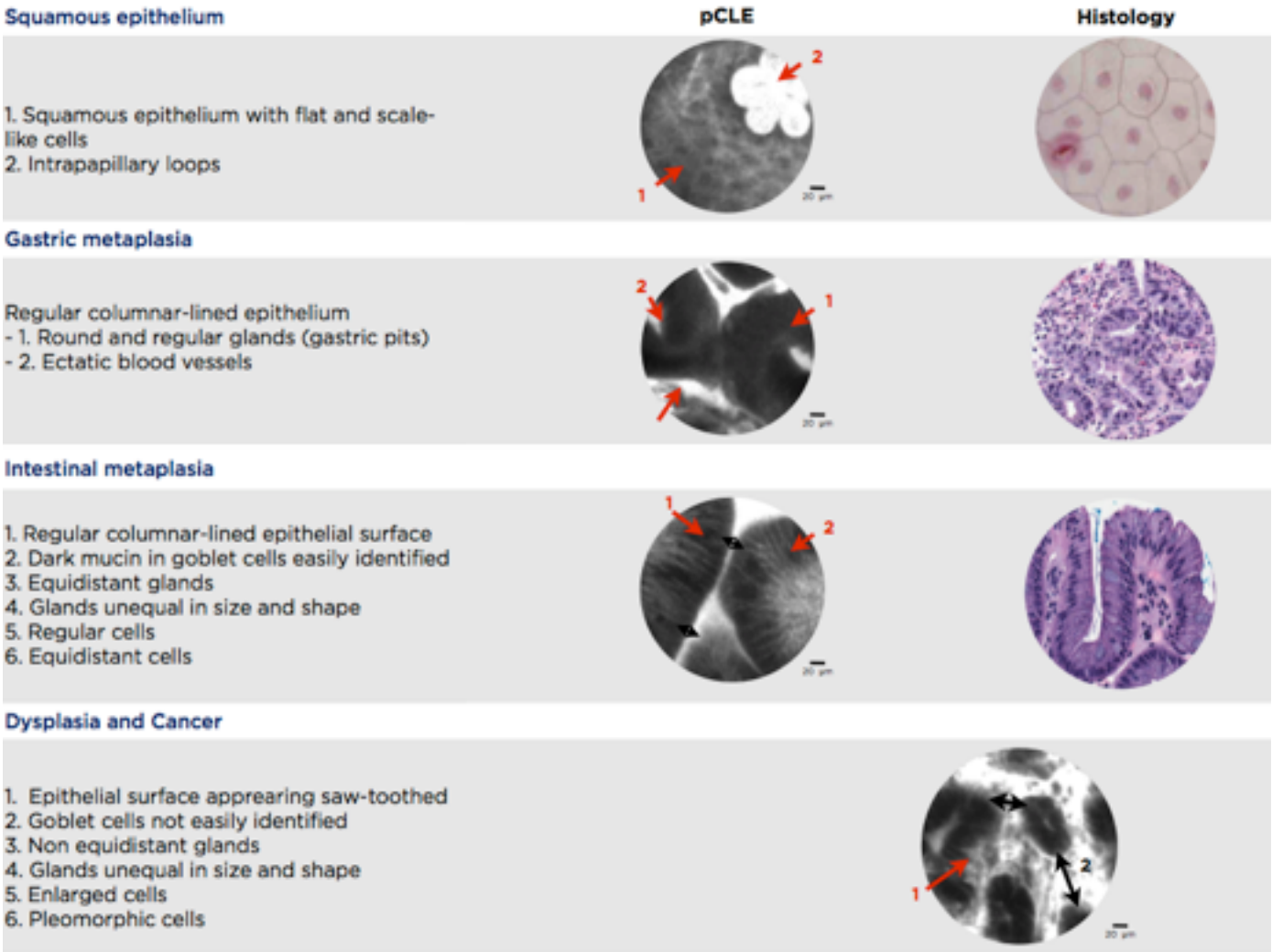

Fig.3: CLE image interpretation criteria for Barrett's esophagus (using GastroFlex UHD)

1.2. Colorectal Diseases

CLE has been assessed for several indications in the colon, from characterization of neoplastic polyps to post-resection assessment of margins, and assessment of inflammation and neoplasia in inflammatory bowel diseases. This technology can be used for classification of tissue at a site already detected by standard or enhanced endoscopy. Ideally a red-flag technique such as chromoendoscopy (NBI or FICE) should be used to screen the mucosa for areas of interest, and these identified areas examined with CLE for a histological diagnosis. A standard classification system, termed the Mainz classification (13) was developed in 2004 for endoscope-based confocal laser endomicroscopy (eCLE) and in 2009 the standardized «Miami» classification was developed for pCLE(Figure 4). Both classifications aim at distinguishing neoplastic from hyperplastic polyps of the colon based on a dark, irregularly thickened epithelial layer characteristic of epithelial dysplasia.

1.2.1. Colorectal Polyps Differentiation

Conventional endoscopy has limited ability to discriminate adenomatous from non-adenomatous colorectal polyps. In daily practice, all identified lesions are routinely removed and sent for histopathology thus inducing costs, and potentially complications, which is a serious limitation. If CLE could reliably predict histology, then it could considerably increase cost-effectiveness and efficiency. This reliance on histology implies that there is an undesirable cost and a small risk of removing polyps with low neoplastic risk.

The decision to leave a polyp in situ and not send it for pathology requires a high accuracy and especially high negative predictive value. Considering up to one-half of colonic polyps are hyperplastic, smaller than 10 mm, and with very low likelihood of malignancy potential (14), selecting the right polyps to remove would greatly improve the practice.

Several groups, including the American Society for Gastrointestinal Endoscopy (ASGE), have provided guidelines for when it would be clinically acceptable to adopt a virtual biopsy approach, in a PIVI initiative (14) . PIVI initiative gathers two strategies for patient management, that are “resect-and-discard” management and “leave behind” management. They set clear thresholds for the accuracy that must be achieved (at least90% negative predictive value for adenomatous polyps and at least 90% accuracy for predicting the correct surveillance interval). They also set clear target lesions (small distal polyps) that are both the most common polyps as well as those at lowest risk for malignant degeneration in the unlikely event that an incorrect diagnosis is made. CLE has therefore been extensively studied to fulfill these recommendations, in regard to colorectal polyps.

De Palma et al (15) assessed the accuracy and IOA of pCLE in colorectal polyps, with a study involving 32 small polyps ranging from 1 to 9 mm, in 20 patients. Lesions were identified using pCLE imaging after white-light endoscopy. pCLE achieved sensitivity and negative predictive value of 100%, specificity of 85%, and positive predictive value of 91% in predicting adenomatous histology, using the final histopathological diagnosis as a reference.

In a study conducted by Buchner et al (16), pCLE was compared with virtual chromoendoscopy in the diagnosis of neoplastic versus non-neoplastic polyps. Sensitivity of pCLE was higher than virtual chromoendoscopy (91% versus 77%, p=0.01) with similar specificity (76% versus 71%). This study included both large (>9 mm) and small polyps. Another study, conducted by Shahid et al (17), in 2011, focused exclusively on small polyps, to define whether the combination of pCLE with NBI may be eligible for a diagnose-and-discard strategy. In this study, pCLE and

NBI were evaluated both independently and in combination. One hundred and thirty polyps <10 mm were evaluated in 65 patients. pCLE had a higher sensitivity than NBI (86% versus 64%, p=0.008) but with lower specificity (78% versus 92%, p=0.027) and similar overall accuracy. When combining pCLE and NBI, limiting the analysis to high-confidence images, sensitivity and negative predictive value were 94% and specificity 97%. These results are significant as they demonstrate the technology meets the PIVI recommended thresholds (90% or greater) for acceptance of a resect-and-discard strategy.

These studies relied on the Miami classification criteria, and did not iterate on those considering the good results they enabled to obtain. The criteria are the following (Figure 4):

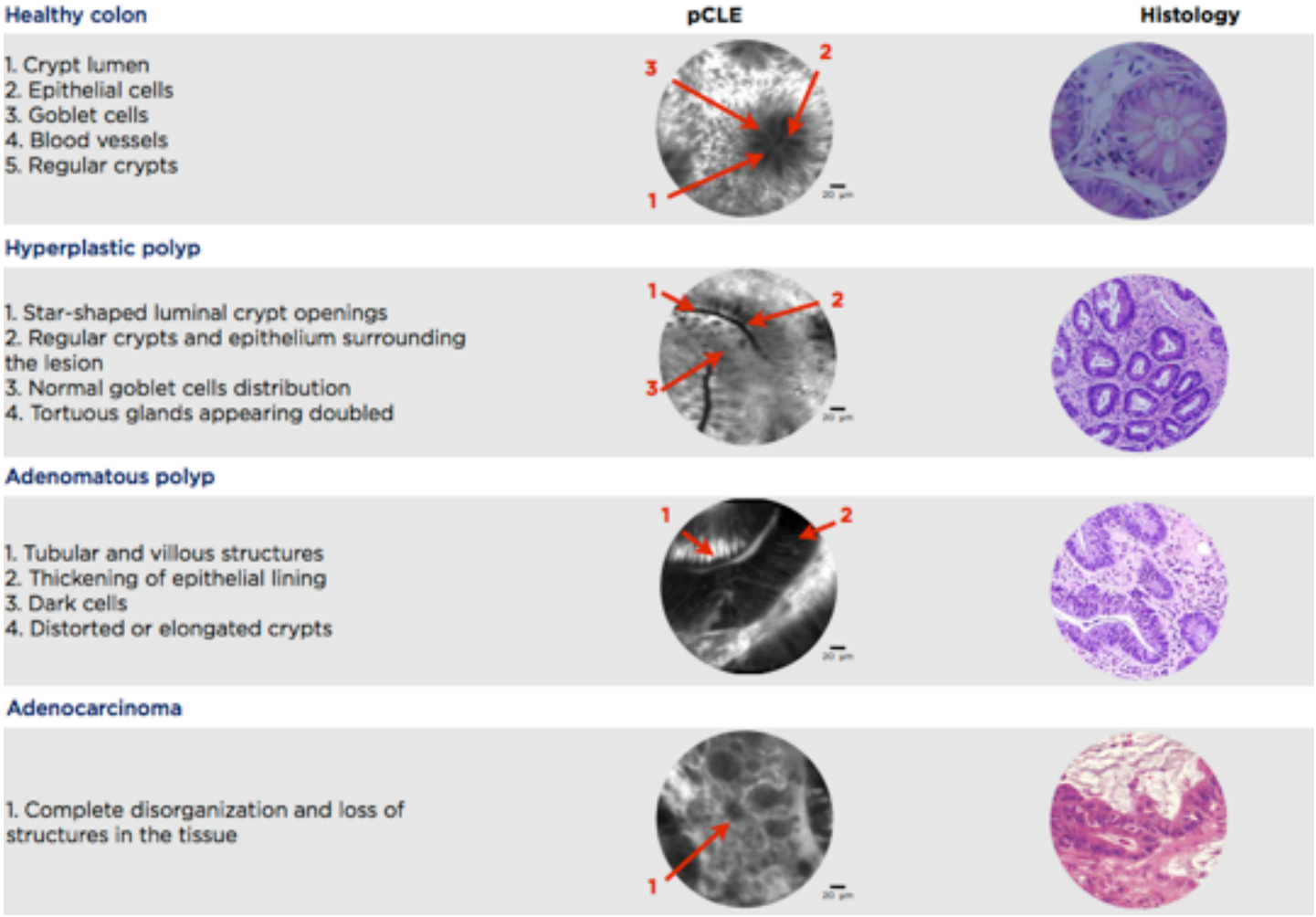

Fig.4: CLE image interpretation criteria for colorectal lesions (using ColoFlex UHD)

• healthy colon: round crypt structures, dark goblet cells, regular and narrow vessels surrounding crypts,

• hyperplastic polyp: star-shaped luminal crypt openings, regular crypts and epithelium surrounding the lesion, normal goblet cells distribution, tortuous glands appearing doubled,

• adenomatous polyp: tubular and villous structures, thickening of epithelial lining, dark cells, distorted or elongated crypts,

• adenocarcinoma: complete disorganization and loss of structures in the tissue.

Another study from Buchner (18) makes a case for the integration of CLE in the clinical routine, thanks to a short learning curve obtained by testing 76 sequences on 11 endoscopists from various pCLE expertise. Accuracy of image interpretation was calculated on a total of 76 pCLE sequences by groups of 20 consecutive sequences in order to evaluate the learning curve for pCLE image interpretation. Accuracy went from 63% for

the first group of sequences to 86% for the last group of sequences, demonstrating a short learning curve for pCLE.

Recently, a meta-analysis published by Wanders et al. (19) established the diagnostic performance of narrowed spectrum endoscopy, autofluorescence imaging, and confocal laser endomicroscopy for the diagnosis of colonic polyps.

Out of the 91 studies included in this analysis, 56 related to NBI, ten to i-scan, 14 to FICE, 11 to CLE, and 11 to autofluorescence imaging. CLE was the only imaging technique shown to meet the PIVI threshold with a NPV>90%, an overall sensitivity of 94% and a specificity of 95%.

1.2.2. Post-EMR Follow-up

In a study by Shahid et al. (20) 129 post-EMR scars were evaluated as neoplastic versus non-neoplastic within one year of initial intervention in 3 centers. The EMR sites and scars were assessed by high-resolution colonoscopy and virtual chromoendoscopy (VCE), either with NBI or FICE, followed by pCLE. pCLE images were reviewed in real-time and later offline, blinded to endoscopic appearance and histology, which was used as gold standard. Overall sensitivity, specificity, positive predictive value, negative predictive value, and accuracy for VCE were 72%, 77%, 49%, 91%, and 77% versus 97%, 77%, 55%, 99%, and 81% for pCLE. In 95 of 129 (74%) scars, both VCE and pCLE agreed with the improvement of many parameters, leading to 100% specificity and negative predictive value. pCLE may therefore improve the sensitivity for detecting residual neoplasia on follow-up colonoscopy after EMR compared with endoscopy with VCE alone. The combination of VCE and pCLE was even more sensitive and specific when the results of both methods agreed. Despite these good results, the predictive positive value is relatively low, which supported a continued need for refining the image quality and classification.

1.2.3. Serrated Adenomas

Serrated adenomas are currently an important topic in the digestive field because of their ambiguous diagnosis and treatment. There are two types of serrated adenomas : traditional serrated adenomas, which typically assume a polypoid appearance, like hyperplastic polyps, and the sessile serrated adenoma (SSA), flat or slightly raised, and located on the proximal colon. If not checked, SSA can turn into malignant lesions, and as such, those lesions have to be removed during colonoscopy.

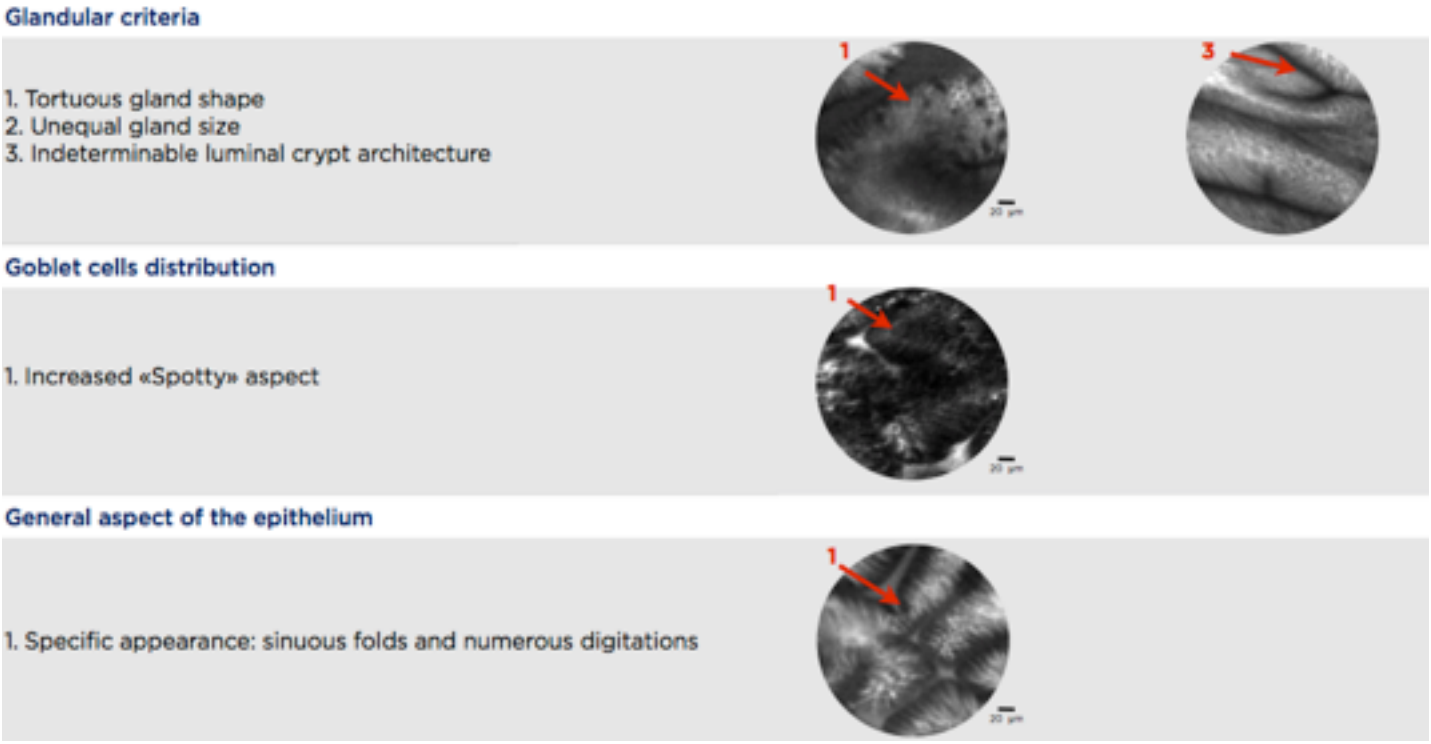

Fig.5: CLE image interpretation criteria for serrated adenomas (using ColoFlex UHD)

However, the distinction between the two types of adenomas remains difficult. (21) first conducted a prospective study with 10 SSAs, to define interpretation criteria which could distinguish them from hyperplastic polyps. The criteria were the following: presence of tortuous glands, of uneven size with indefinite lumen (glandular criteria), variation in the goblet cell distribution with a "spotty" aspect of the epithelium, and presence of numerous sinuous folds and projections, achieving an "undulating" aspect of the surface (general aspect of the epithelium) (Figure 5). A validation study was then conducted by Leblanc et al. (22) to assess these criteria, by identifying 11 Serrated adenomas (SSAs). The glandular criteria, goblet cell distribution, and general aspect of the epithelium were observed in over 82%, 100% and 100% of the adenomas, respectively. The criteria looked promising for the differentiation of hyperplastic polyps and SSAs but their performance characteristics and predictive values need to be prospectively evaluated in a larger setting.

1.3. Inflammatory Bowel Diseases

The risk of developing digestive cancer increases from 0.5% to 1% every year after 8 years of disease for patients with ulcerative colitis (UC) or Crohn's disease (CD). The differentiation between Dysplasia-Associated Lesional Mass (DALM) and Adenoma-Like Mass (ALM) is not reliable with conventional endoscopy (23). As patients with UC or CD have an increased risk of developing dysplasia, guidelines recommend colonoscopic surveillance including systematic biopsy sampling, with multiple random biopsies. Taking many biopsies is time consuming, carries a low but non negligible risk of secondary hemorrhage, and has only moderate sensitivity for neoplasia detection especially when random biopsies are taken (24). CLE during endoscopy surveillance procedures of patients with IBD has shown high agreement with histological findings (25). The best combination in UC surveillance is chromoendoscopy plus CLE, as

chromoendoscopy is the gold standard to detect suspicious areas that can then be examined by CLE to confirm intraepithelial neoplasia and guide immediate therapy. To date, various studies have addressed the potential of CLE in the surveillance of IBD patients, highlighting the role of this technique in assessing the extension of disease, targeting biopsies, and improving the early detection of dysplasia. CLE has also been used by physicians to go beyond the clinical symptoms, by assessing the mucosal status at the microscopic level, and recommending adapted treatment protocols. It could help define when the patient reaches remission through complete mucosal healing.

Kiesslich et al. (26) used the combination of chromoendoscopy, (to detect flat or suspected lesions), and CLE(to diagnose neoplastic and non-neoplastic tissue), in a randomized study on 161 patients. They detected 4.75 times more neoplastic lesions, and the number of biopsy specimens was reduced by half. A case report from De Palma et al. (27) used pCLE to diagnose DALM. This technique could therefore provide gastroenterologists with important information for selecting patients that are suitable for immediate endoscopic resection versus referral for pan-proctocolectomy. Moreover, Neumann et al. (25) demonstrated a short learning curve for pCLE and high agreement between pCLE and histopathology findings. Another study, from Li et al. (28) assessed the potential of CLE in the grading of colitis. CLE could indeed provide information equivalent to conventional histology, differentiating between active and nonactive UC patients during ongoing endoscopy. The inflammation activity assessment includes crypt architecture, cellular infiltration, and vessel architecture.

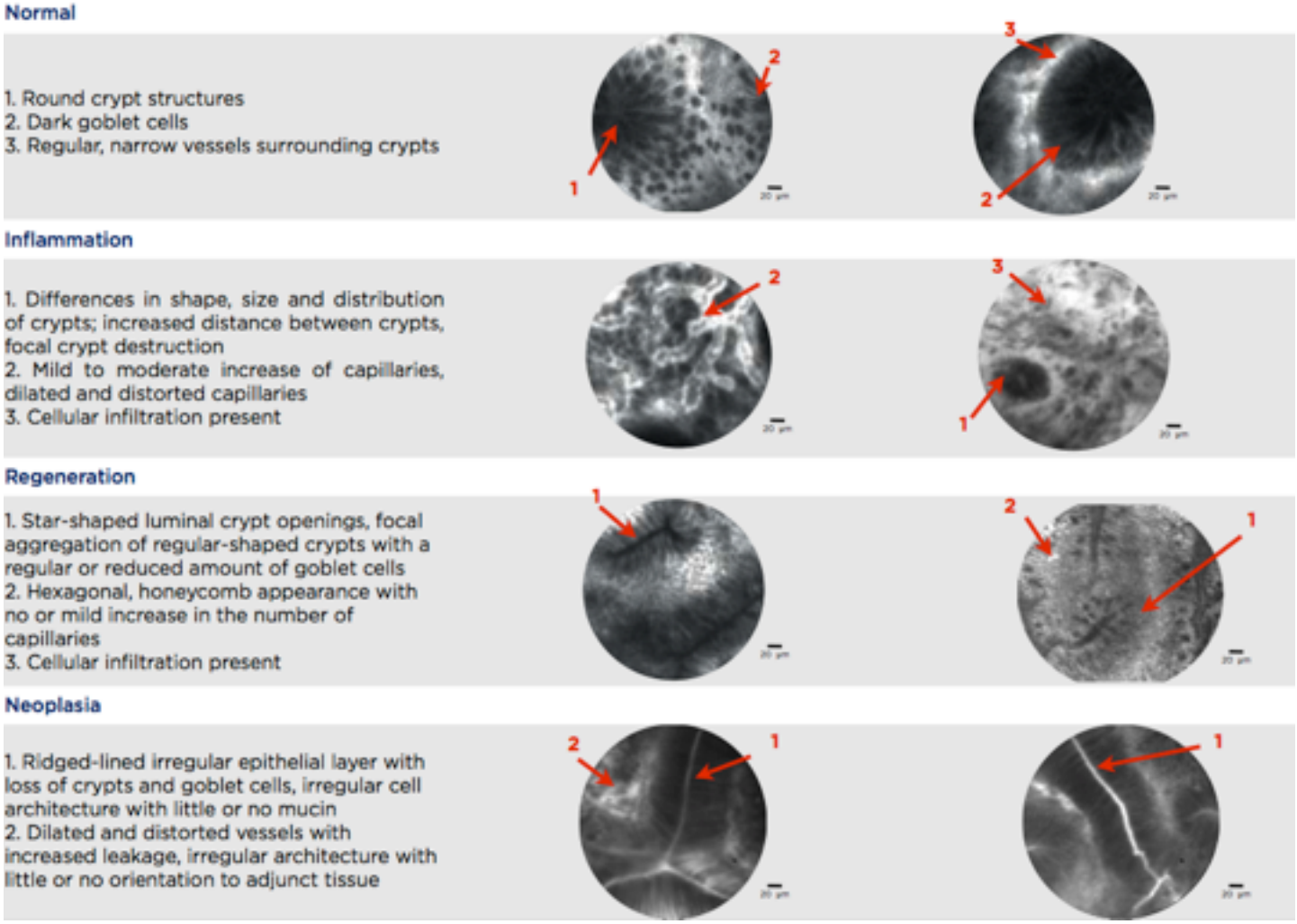

Fig.6: CLE image interpretation criteria for inflammatory bowel disease (using ColoFlex UHD)

There is at present no worldwide accepted classification of CLE images in IBD, but many authors refer to the classification established by Kiesslich et al. (26) in 2005 for ulcerative colitis, which differentiates normal mucosa, regeneration, neoplasia, and inflammation, using crypt architecture, cellular infiltration, and vessel architecture (Figure 5).

As for Crohn's disease, Neumann et al. (29) has developed a new score, the Crohn's Disease Endomicroscopic Activity Score (CDEAS) to assess CD activity in vivo. Their study included 54 patients, with 18 patients without IBD who served as controls. The CDEAS consists of six parameters, corresponding to interpretation criteria, identified as characteristic for active CD compared with inactive CD and controls:

- crypt number (increased or decreased)
- crypt distortion
- micro erosions
- cellular infiltrate
- vascularity
- number of goblet cells (increased or decreased)

By assignment of one point for each given parameter, the score ranges from 0 to 8. The median scores were 2 and 5 for patients with quiescent and active CD respectively. The CDEAS strongly correlates with C-reactive Protein (CRP), an already established biological inflammatory marker of CD. Therefore, CDEAS has a potential for assessment of CD activity.

The same author (30) recently presented a study, which aimed at defining and assessing a new classification for pCLE in IBD. First, a post hoc review of 25 pCLE video sequences was performed in order to accomplish a new classification system based on different vessel and crypt categories.Using this refined classification, the sensitivity, specificity and accuracy for predicting histological inflammation in macroscopically non-inflamed mucosa were 94%, 81%, and 87%, respectively. Positive and negative predictive values were 82% and 94%, respectively. This new classification allows prediction of microscopic inflammation in macroscopically non-inflamed mucosa.

Buda et al. (31) reported their results on a cohort of 38 patients (19 CD patients in remission and 19 control patients) and showed that *in vivo* intramucosal changes detected by confocal endomicroscopy in ulcerative colitis remittent patients can predict disease relapse. This observation may have further implications for disease management and medical treatment.

1.4. Gastric Diseases

Gastric cancer is the second leading cancer-related death worldwide. Its incidence is higher in Asia than in western countries. Atrophic gastritis, and intestinal metaplasia, following Helicobacter Pylori infection, represent pre-cancerous conditions. In order to diagnose these types of lesions, current guidelines recommend the Sydney protocol, which consists in 5 physical biopsies taken at specific locations in the stomach. These random biopsies are not specifically targeted leading to a poor diagnostic yield for dysplastic lesions.

These consecutive stages of pre-cancerous conditions have been studied with CLE in the last few years. A study conducted by Wang et al on 118 patients (32) achieved a high sensitivity and specificity for the diagnosis of Helicobacter Pylori infection and Helicobacter Pylori-associated glandular atrophy by CLE (82.9% and 90.9%, as well as 92.9% and 95.2%, respectively). In another study, conducted on 25 patients, Guo et al. (33) obtained high sensitivity and specificity for the diagnosis of gastric intestinal metaplasia (GIM):98.13% and 95.33% for CLE vs. 36.88% and 91.59% for WLE. Li et al. (34) evaluated the diagnostic value of CLE versus WLE for gastric superficial lesions in a 2-phase study: a first study conducted on 182 patients to define interpretation criteria for GIM, gastric intraepithelial neoplasia (GIN) and cancer, and a second study conducted on 1572 patients to

validate these criteria by assessing the efficacy of CLE. The sensitivity and positive predictive value of CLE for the diagnosis of gastric superficial cancerous lesions were significantly higher than the sensitivity and PPV of WLE (88.9% and 85.3% versus 72.2% and 41.6%), with similar specificity and NPV. Lim et al. (35) evaluated 75 lesions of GIM on 12 patients with WLE, NBI, and pCLE. The sensitivity, specificity, and accuracy of pCLE were 88.6%, 90.3%, and 89.3% versus 27.3%, 96.8%, and 56% for WLE respectively. pCLE, as well as NBI, was therefore superior to WLE for the diagnosis of GIM. A learning curve study from Pittayanon et al. (36) showed that after a short session of training and quiz on GIM by pCLE, novice interpreters can achieve a high level of reading accuracy with substantial level of IOA. They also maintain their good reading skills once they achieve the high reading accuracy.

Bok et al. (37) aimed at determining the accuracy of pCLE compared with conventional forceps biopsy by using histopathology results after endoscopic resection as a reference, and comparing real-time in vivo pCLE diagnosis with that of blinded offline pCLE diagnosis and offline interobserver agreement. *In vivo* real time pCLE provided a 90.7% accuracy versus 85.2% for conventional biopsies, with an excellent agreement between pCLE and histopathology (kappa=0.824), showing that pCLE has the potential to compensate for the inherent limitations of a conventional endoscopic biopsy

There is no established classification for gastric lesions, but similar criteria categories (glandular architecture, cell morphology, and vessel architecture) have been used for the studies above, to define several stages of pre-cancerous lesions: These criteria are the following:

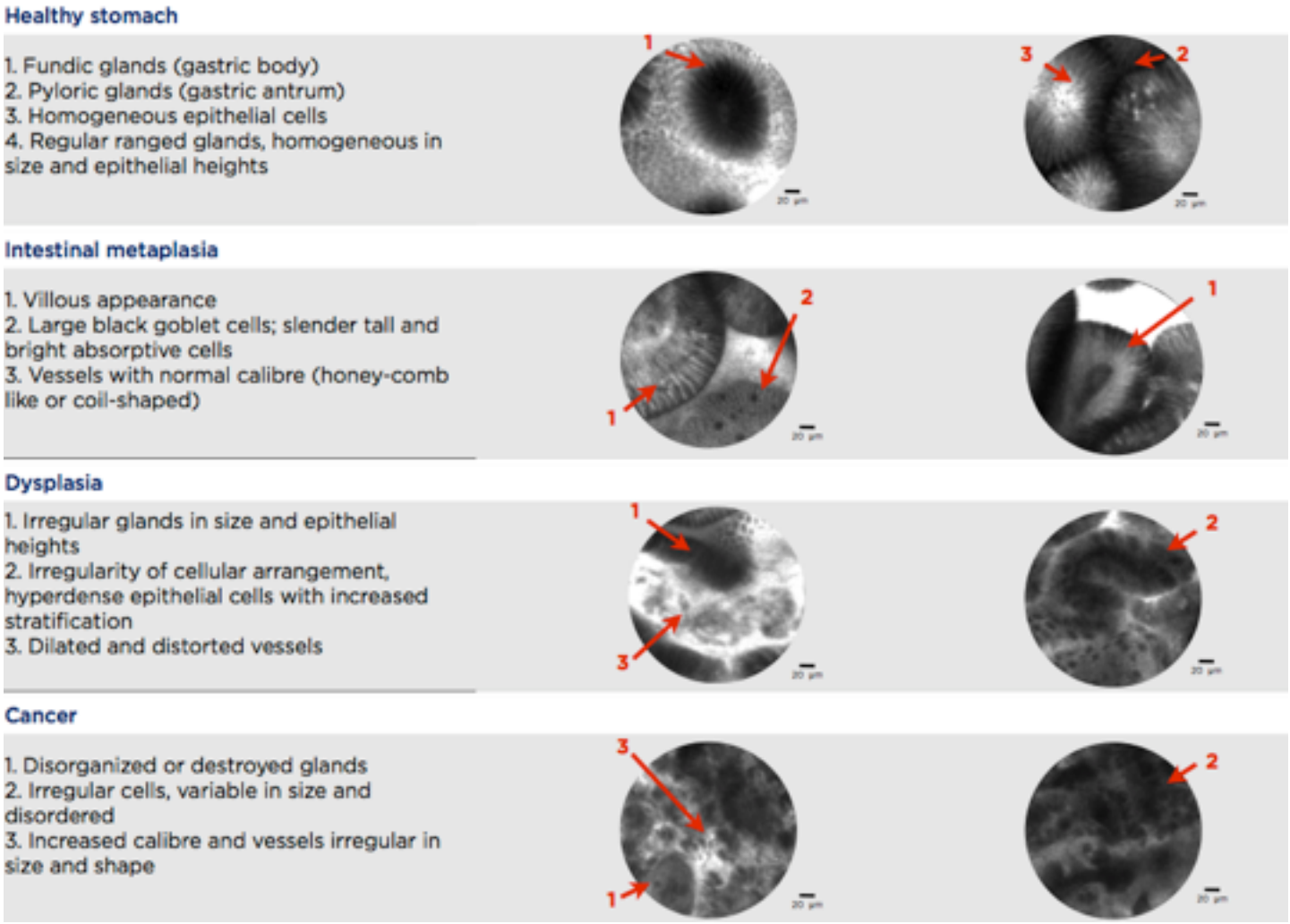

Fig.7: CLE image interpretation criteria for Gastric Diseases (using GastroFlex UHD)

• healthy stomach: fundic glands are distinguished from pyloric glands; both types of glands are characterized by homogeneous epithelial cells, and regular ranged glands; homogeneous in size and epithelial heights;

• intestinal metaplasia: villous appearance; large black goblet cells; slender tall and bright absorptive cells, normal calibre,

• dysplasia: irregular glands in size and epithelial heights, irregularity of cell arrangement, hyperdense epithelial cells with increased stratification, dilated and distorted vessels,

• cancer: disorganized or destroyed glands, irregular cells, variable in size and disordered, increased calibre; and vessels irregular in shape.

These criteria are presented in Figure 7.

In a study on 20 patients, Lim et al. (38) demonstrated that pCLE was superior to AFI and WLE for diagnosing GIM. A total of 125 patients in 20 sites were examined. For diagnosing GIM, real-time pCLE had better sensitivity (90.9 vs. 37.9%, $p < 0.001$) and accuracy (88.0 vs. 64.8%, $p < 0.001$) compared with WLE. Sensitivity (90.9 vs. 68.2%, $p = 0.001$), specificity (84.7 vs. 69.5%, $p = 0.042$), and accuracy (88 vs. 68.8%, $p < 0.001$) of real-time pCLE were better than AFI. Sensitivity, specificity, and accuracy of real-time pCLE and mNBI for diagnosing GIM were similar. Off-site pCLE had significantly better accuracy for diagnosing GIM compared to WLE, AFI, and mNBI. Off-site pCLE had superior specificity (94.9 vs. 84.7%, $p = 0.031$) and accuracy (95.2 vs. 88.0%, $p = 0.012$) compared with real-time pCLE.

A recent randomized study by Li et al (39), aimed at comparing the diagnostic yield of GIM between confocal laser endomicroscopy (CLE) and white light endoscopy (WLE) in a cohort of 168 patients (in 2 arms). On a per-biopsy analysis, CLE-targeted biopsy gave a significantly higher diagnostic yield of GIM compared with WLE and standard biopsy, at 65.70 % (113 /172 biopsies) versus 15.73 % (81 / 515 biopsies) ($P < 0.001$). Use of CLE-guided biopsy decreased by 68 % the mean number of biopsies required per patient.

2. Digestive Ductal Indications: Biliary Diseases

Prognosis and therapy guidance for pancreaticobiliary cancers require accurate diagnosis and staging. Management of patients with indeterminate pancreaticobiliary strictures is complex, despite advanced imaging and tissue sampling methods. Even when several sampling methods such as brushing biopsy or aspiration are combined, the outcomes remain suboptimal, with sensitivity results of 54% to 71%, but specificity of 100% (40, 41). There is a critical need to improve diagnostic accuracy and enable a more tailored approach to patient care, in order to reduce unnecessary interventions and provide patients with earlier diagnosis.

pCLE is currently the only endomicroscopic technique enabling an in vivo and real- time characterization of indeterminate pancreaticobiliary strictures, with a dedicated probe thin enough to be introduced through the working channel of a cholangioscope or inside a catheter used during an ERCP procedure. pCLE has the potential to overcome the limited accuracy of current diagnostic method, by providing real-time, microscopic images of the bile duct epithelium. This visualization in its finest details and in real-time, provides key histological information that is missing most of the time.

A prospective observational multicenter registry (Cellvizio ERCP registry) has been conducted with 102 patients (42). This study aimed at documenting utility, performance and accuracy of real-time pCLE diagnosis

compared with histopathology, and at validating the Miami classification. pCLE obtained significantly higher sensitivity and NPV than index pathology (98% and 97% versus 45% and 69%, respectively). This resulted in an overall accuracy of 81% for pCLE compared with 75% for index pathology. The combination of ERCP and pCLE was also more accurate than the combination of ERCP and tissue sampling (90% versus 73%, respectively). This supports supplementing ERCP with pCLE, rather than biopsies or brushings.

A second publication by Meining et al. (43) presents the classification developed and validated during the same registry for the interpretation of pCLE image obtained in the pancreaticobiliary system. The investigators tested the criteria developed in the ERCP registry through a blinded consensus, reviewing 112 randomized pCLE videos from 47 patients. Inter-observer variability was assessed in 42 patients. The characteristics most suggestive of malignancy included the following: thick white bands (>20 microns), thick dark bands (>40 microns), dark clumps, or epithelial structures. Combining two or more criteria significantly increased the sensitivity, and predictive values. Combining them all provided sensitivity, specificity, PPV and NPV of 97%, 33%, 80% and 80% compared with 48%, 100%, 100%, and 41% for standard tissue sampling methods. Inter-observer variability was moderate for most criteria. These criteria have been confirmed by others who described similar criteria of benign versus malignant biliary epithelium (44, 45)

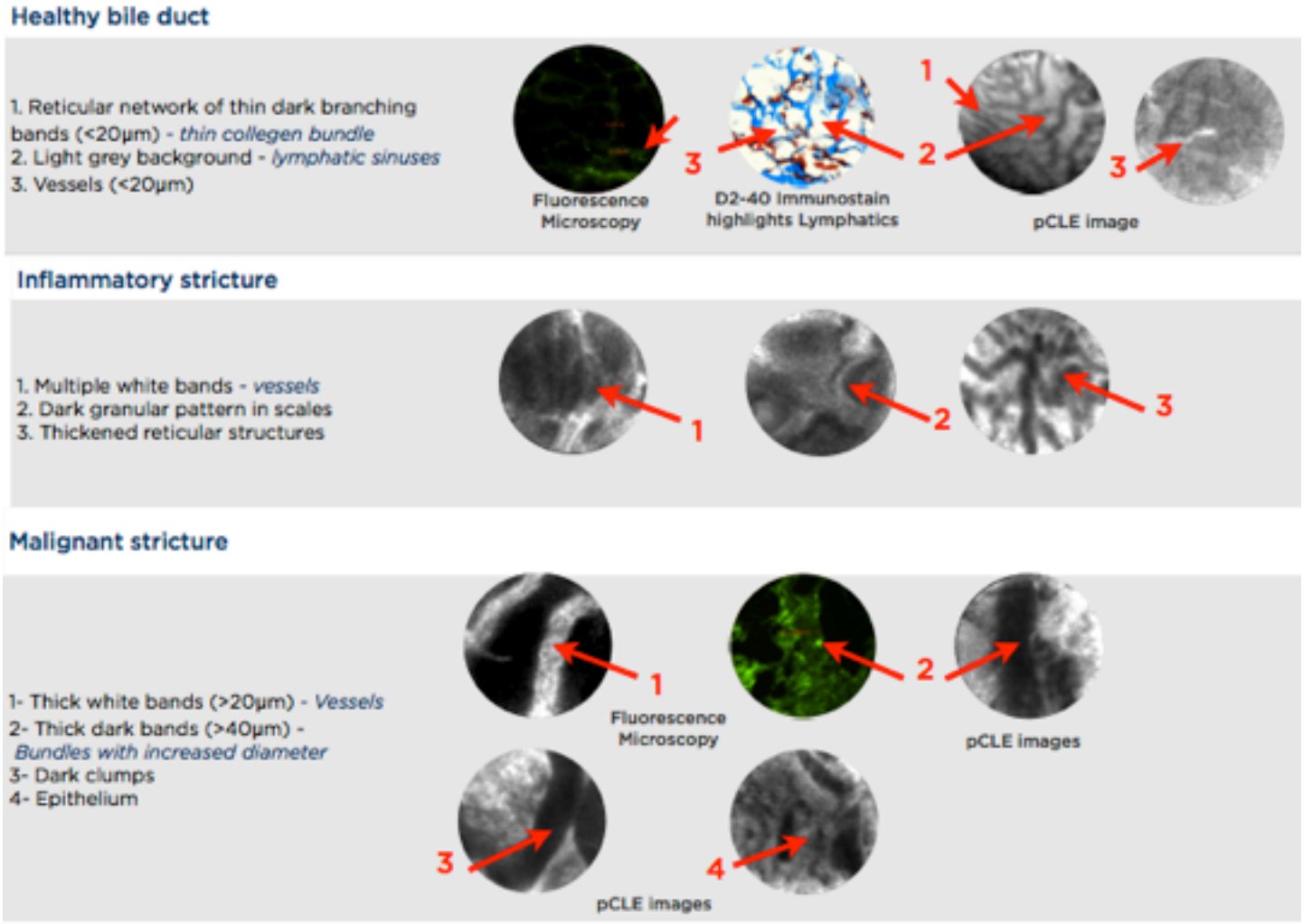

Fig. 8: CLE image interpretation criteria for Bile duct lesions (using CholangioFlex)

This classification demonstrates a limited specificity which may be explained by a confusion between (pre-)cancerous tissue and inflammatory induced changes, observed after stenting or in other pathology-related strictures such as Primary Sclerosing Cholangitis (PSC).

A retrospective study was conducted in order to refine the Miami classification to better characterize inflammatory strictures (46). The investigators first established new criteria specific to inflammation by reviewing 60 sequences from the registry, and then evaluated the diagnostic efficacy of pCLE with these new criteria on 40 additional sequences. The following criteria were defined : thickened reticular structures, dark granular pattern with scales, multiple thin white bands, and increased spaces between scales (>20microns).

An international, prospective, multicenter study (FOCUS) is ongoing to validate these novel criteria prospectively and assess the impact of the technique on patient management.

A comprehensive analysis of biliary pCLE imaging and histologic correlates utilizing a novel frozen sectioning protocol has recently been conducted by Benias et al (47). This study aimed at reproducing pCLE images ex-vivo, which has allowed for a multimodal assessment. The corresponding findings suggest that thin dark bands forming a reticular pattern may identify submucosal collagen network;previously termed “white bands” are in fact lymphatic sinuses and small blood vessels; black bands” are collagen fibrils, which structurally support this network, and predictably increase in diameter within pathologic specimens.

Optical biopsy has been shown to achieve a high technical success rate in patients with primary sclerosing cholangitis with dominant biliary strictures (48). In a single center study conducted with15 patients, endomicroscopy provided a sensitivity of 100%, specificity of 61.1%, PPV of 22.2% and a PPV of 100% for the characterization of neoplasia. If verified in larger prospective studies, the technology may be utilized to risk stratify dominant strictures in patients with PSC.

3. Solid Organs

3.1 Pancreatic Cysts

Pancreatic cancer is the fourth leading cause of cancer-related death in the United States. This disease is associated with a high mortality rate with: 5-year survival rate estimated to be 4%. This is mainly due to the fact that the disease is often discovered at an already advanced disease state, which carries a dismal prognosis (49). Early detection and exclusion of metastases followed by prompt resection of the tumor promises the best chances for survival. Pancreatic lesions include pancreatic cysts, and pancreatic solid masses (or tumors).

The principle of needle-based Confocal Laser Endomicroscopy (nCLE) is to image organs within or adjacent to the GI or respiratory tracts with a miniprobe inserted through an endoscopic needle. The fundamental technology as well as the principle of operation of nCLE are substantially similar to pCLE,but the miniaturization enables through-the-needle access. It is expected to help differentiate the different types of lesions, especially for cysts, leading to a better patient management. Real-timemicroscopic tissue informatio in vivo during an Endoscopic UltraSound Fine Needle Aspiration (EUS-FNA) procedure may allow for better differentiation between mucinous and non-mucinous cysts.

A study conducted by Konda et al. (50) helped to demonstrate the technical feasibility, define a precise imaging protocol and assess the safety of the procedure with prototype

probes. Images were obtained from pancreatic cysts and solid masses. Typical patterns of pancreatic cysts could be observed and were suggestive of the potential of this new imaging technique for solid organs.

A second nCLE study, INSPECT (in vivo nCLE Study in the Pancreas with Endosonography of Cystic Tumors) (51, 52), aimed at defining interpretation criteria for the differentiation between mucinous and non-mucinous cysts, and assessing the safety of the procedure. From the 65 patients enrolled in the study, the first 27 cases were reviewed to define a list of descriptive criteria for pancreatic cysts.

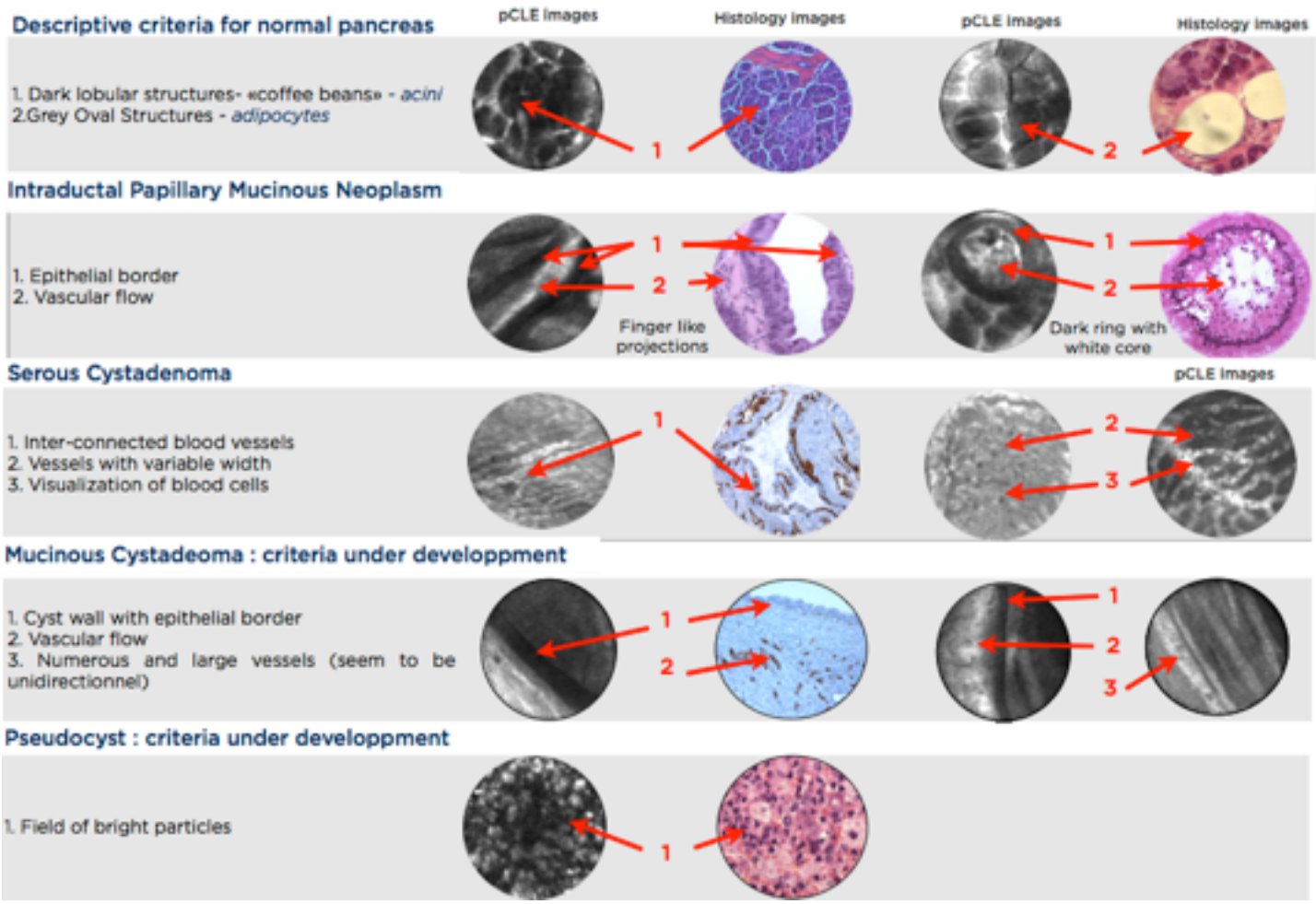

Fig.9: CLE image interpretation criteria for Pancreatic cysts (using the AQ-Flex 19)

These criteria were divided into three categories :

• epithelial structures: finger-like projections; dark ring with white core; dark aggregates of cells; glandular structures,

• pancreatic and peri-pancreatic structures: dark lobular structures;grey oval structures, white band; dark bands, ultra thin straight bright grey bands;

• floating luminal and other structures: small black floating particles; heterogeneous-sized bright particles; large dark round homogeneous floating structures; bright uniform particles in clusters.

The accuracy results of nCLE obtained in this study are based on one main criterion

(papillary projections), which is directly linked and specific to IPMN cysts, and helped therefore identify mucinous cysts. These results show that off-line blinded nCLE has a sensitivity of 59% for the detection of mucinous cysts and a specificity of 100%, and that nCLE has a better diagnostic yield than both CEA and cytology (42% versus 29% and 30%, respectively). When the three methods are combined, the yield is 55%.

In the DETECT study (53), 30 patients with pancreatic cysts were enrolled. A combination of direct visualization using a through-the-needle fiber optic probe (Spyglass) and nCLE (interpreted using criteria defined in the INSPECT study) was used to differentiate mucinous vs. non-mucinous cysts. Mucinous cysts were characterized by the following identified specific features: mucin on Spyglass images, epithelial structures on nCLE, and CEA level superior to 192. Sensitivity for mucinous cysts using Spyglass alone, nCLE alone, and both techniques was 90%, 80%, and 100%, respectively on 18 high certainty cases and 71%, 77% and 88% on 30 all cases

A multicentric, interventional prospective study (CONTACT 1) was conducted in France at 3 centers. 31 patients with a pancreatic cystic lesion of unknown diagnosis were prospectively included (54). The investigators were able to identify a nCLE criterion characteristic of serous cystadenoma (SCA). They correlated their findings to the pathology of archived specimens. The superficial vascular network criterion, visualized on nCLE, corresponds to a dense and subepithelial capillary vascularization only evidenced in SCA in pathological specimens. Accuracy, sensitivity, specificity, Positive Predictive Value and Negative Predictive Value of this criterion for the diagnosis of SCA were 87%, 69%, 100%, 100% and 82%, respectively. Interobserver agreement was substantial (K=0.77). This new nCLE criterion seems highly specific for the diagnosis of SCA. The visualization of this criterion could have a direct impact on the management of patients by avoiding unnecessary surgeries. The prospective validation of these findings is ongoing.

3.2 Pancreatic Masses

Interim results of a French multicentric study on the use of nCLE images in pancreatic masses (CONTACT study) (55) were recently presented.

The definition of the preliminary interpretation criteria was done via a consensus review of nCLE pancretic mass cases , by 4 investigators and one pathologist.

This preliminary classification of nCLE images obtained in pancreatic masses could help in the differentiation of malignant tumors from normal pancreatic tissue. The second phase of the study should enable validation of this classification and refinement via the identification of new criteria. nCLE could therefore facilitate the diagnosis of these lesions, by bringing in vivo microscopic information, in real-time. The second phase of the study is ongoing and the classification has been refined to better distinguish between the different types of pancreatic masses.

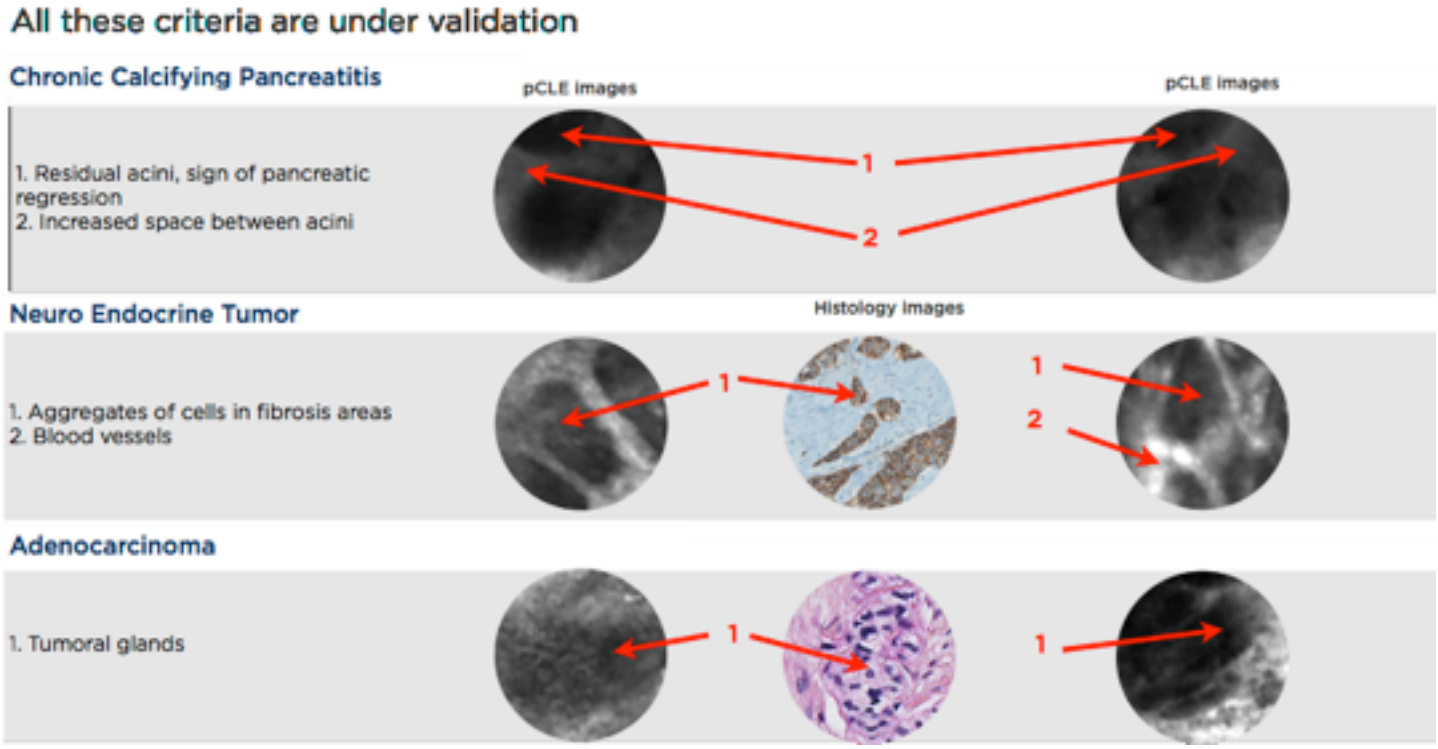

Fig.10: CLE image interpretation criteria for Pancreatic masses (using the AQ-Flex 19)

These criteria are the following:

- chronic calcifying pancreatitis: residual acini which represent pancreatic regression. larger fibrosis area between residual acini than for healthy acini tissue
- neuro-endocrine tumor: aggregates of cells in fibrosis areas, blood vessels
- adenocarcinoma: tumoral glands (dark clumps), cribriform patterns

3.3 Lymph Nodes

A feasibility study evaluating 9 lymph nodes, has shown (5 mediastinal, 4 celiac), easily identifiable differences between confirmed malignant lymph nodes and inflammatory lymph nodes. Final diagnosis was 7 malignant lymph nodes (2 from gastric cancer, 2 from colon cancer, 2 from cardia carcinoma, and 1 from lung cancer) and 2 inflammatory lymph nodes. Inflammatory lymph nodes were characterized by the presence of diffuse small cells into a homogeneous stroma without vascularization. In malignant lymph nodes, nCLE showed some glandular structures with dark cells and vascularization.

These criteria, which have been recently more precisely defined and correlated with histological structures are presented in Figure 11.

Further clinical data is needed to confirm the potential of nCLE to differentiate inflammatory and malignant lymph nodes.

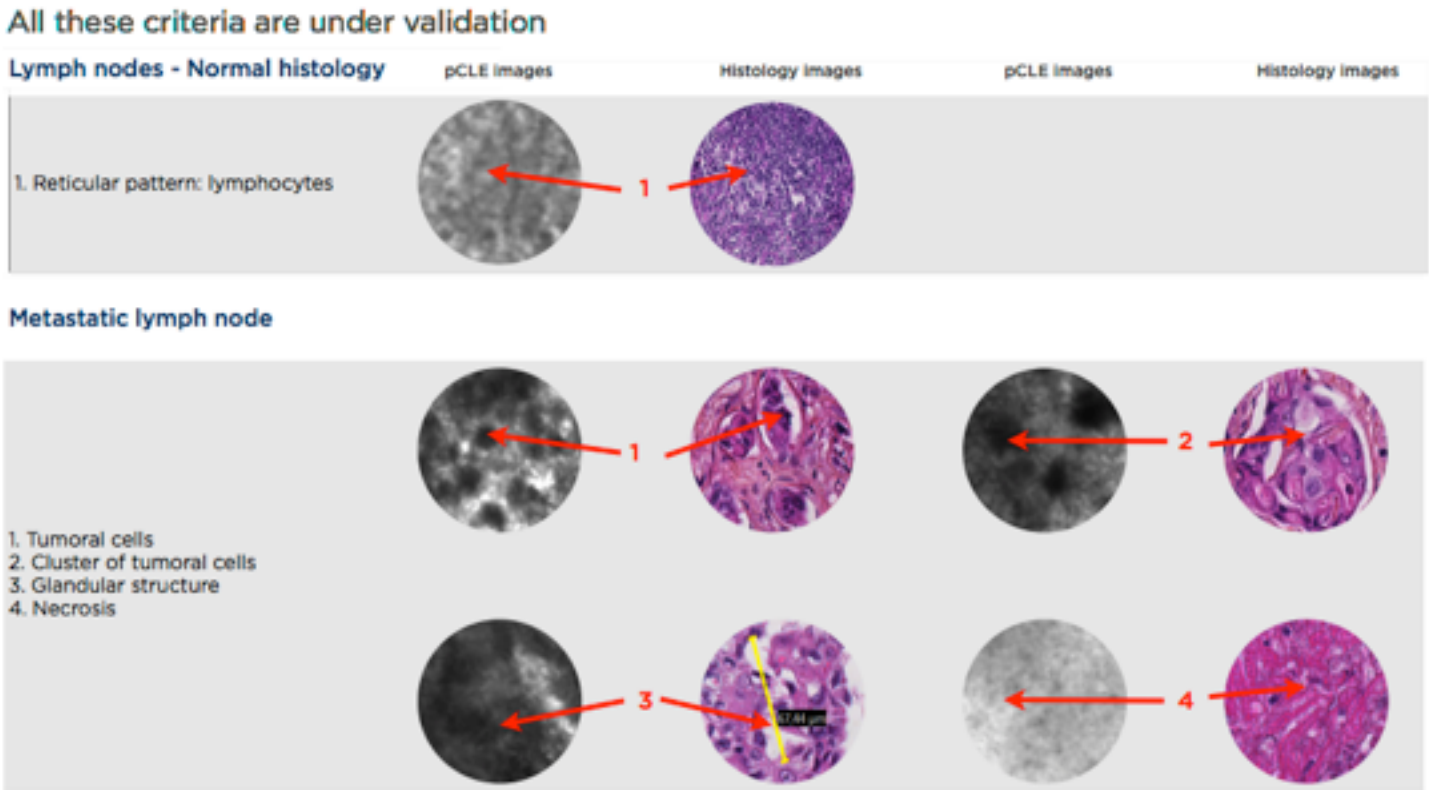

Fig.11: CLE image interpretation criteria for Lymph Nodes (using the AQ-Flex 19)

4. Urology: Bladder Diseases

pCLE has also been studied in the urinary tract, where the technology has potential for the characterization of neoplastic conditions.

K. Wu et al. (56) suggested diagnostic criteria that could be used for pCLE image interpretation in urology and demonstrated the safety profile of the procedure and of fluorescein administration. They recruited 66 patients scheduled for transurethral resection of bladder tumor (TURBT) and 2 patients scheduled for nephrectomy, and performed white-light cystoscopy (WLC) followed by pCLE in the area of interest. Confocal images were then treated using a mosaicing post hoc algorithm, to better evaluate tissue microarchitecture. Hematoxylin and Eosin tissue sample analysis was considered as gold standard. They identified key features in several organs (kidney, ureter, bladder, prostate, and urethra) of the urinary tract. After real-time imaging, some some high quality sequences were selected, and associated mosaics were created. Then, two pathologists reviewed and compared the sequences with Hematoxylin and Eosin stains, to select diagnostic criteria representative of different types of lesions, that are reproductible and easily recognizable. Normal bladder urothelium was shown to present the following criteria: polygonal umbrella cell layer, uniform smaller intermediate cell layer, and capillary networks with erythrocytes in the lamina propria (Figure 11). Low grade urothelial carcinoma (LGUC) was characterized by densely packed urothelial cells that are homogenous and monomorphic, cellular papillary structures and fibrovascular stalks. Criteria observed for high grade tumors are the same as for LGUC, but with greater disorganization. Carcinoma In Situ (CIS) is among the most challenging lesions to diagnose because it is difficult to differentiate from benign conditions. The study showed that CIS presented more pleomorphic cells compared to inflammatory conditions, as well as indistinct cell borders and extensive acellular areas. Inflammation criteria were demonstrated to be loosely arranged aggregations of smaller monomorphic cells in the lamina propria and local recruitment of inflammatory cells. Some images were taken in renal areas, with renal cortex characterized by tubular structures consistent with convoluted renal tubules and renal pelvis, and proximal ureter demonstrating urothelial cells and lamina propria similar to bladder urothelium.

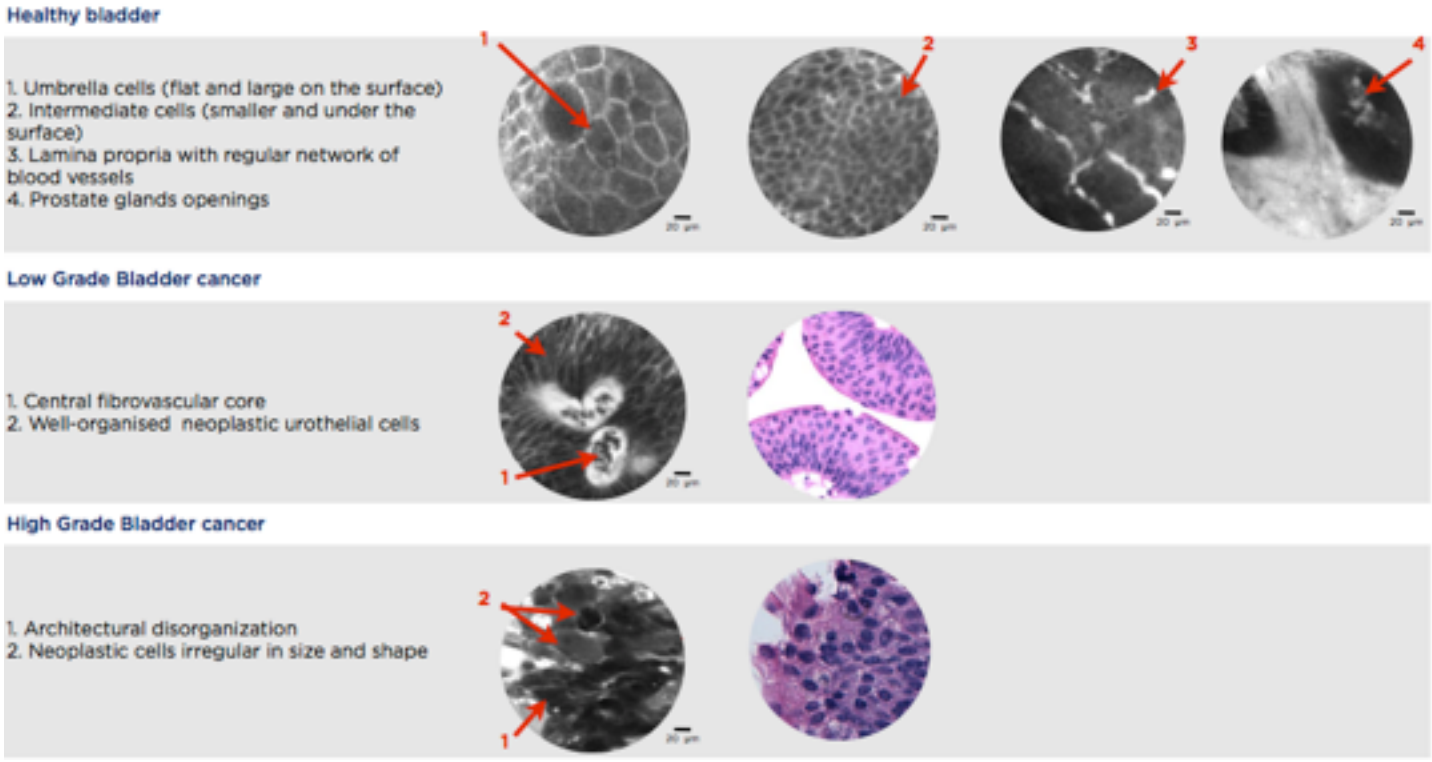

Fig.12: CLE image interpretation criteria for Bladder Cancer (using the Cystoflex UHD)

According to this study, when combined with other macroscopic imaging technologies as fluorescence cystoscopy or NBI, pCLE could be useful to better characterize lesions of the urinary tract, particularly CIS which represents a big challenge with the current pathologic analysis.

In another study by Liu et al (57) published in 2012, 57 patients scheduled to undergo TURBT underwent WLC followed by pCLE, and histologic confirmation of resected tissue. The accuracy in the diagnosis of high grade tumors was increased with pCLE alone, compared to WLC (67 % versus 52%), and the combination of pCLE and WLC obtained a 100 % accuracy for the diagnosis of low grade tumors. As an adjunct to WLC, pCLE may offer the potential for real-time bladder cancer grading.

Bonnal et al (58) studied the association of pCLE with photodynamic diagnosis (PDD) in an ex vivo pilot study. The combination of both techniques demonstrated the usefulness of hexyl aminole- vulinic acid for guiding blue-light CLE. PDD does not provide any histologic evidence but allows definition of areas of interest for CLE.

A study by Chang et al. (59) evaluating IOA demonstrated that pCLE is an adoptable technology for cancer diagnosis in novice CLE observers following a short training. The corresponding IOA was evaluated as moderate and the corresponding diagnostic accuracy was similar to WLC alone. Experienced CLE observers may be capable of achieving substantial levels of agreement for cancer diagnosis that is higher than with WLC alone.

5. Respiratory Diseases

Image interpretation in the healthy lung (from the trachea down to the alveoli) has been defined by Thiberville et al originally (60) and can be summarized in Figure 13.

pCLE images in the lung can be obtained without an exogenous fluorophore, as they display the autofluorescence of the elastin fibers present in the bronchial basement membrane, as well as the acinar distal elastic network. Alveolar macrophages are visible in active smokers, presumably because they contain tobacco tar.
In the proximal lung, the elastin fibers have different structures and density from the trachea down to the bronchioles. They present a dense and homogeneous pattern with visible gland openings in the trachea, while the fibers become more visible individually and are arranged either in parallel or perpendicular bands in the bronchi. This pattern becomes looser in the bronchioles,

and the imprint of the muscular fibers can be recognized around the distal membranous bronchioles. Respiratory bronchioles are usually bypassed, because of the respective size of the 1mm miniprobe and distal airways. Instead, penetration into the acinus is obtained through the parietal wall of a distal bronchiole, resulting in various images of the acinar elastin network, in relation to the angle of penetration of the miniprobe into the acinus.
In the distal lung, after entering the alveolar territory, the elastin fibersand alveolar walls can be recognized as round and homogeneous structures. Some blood microvessels can be identified, due to elastin presence in their walls. Macrophages can be seen in various densities depending on the degree of smoking history of the patient.In the field of bronchoscopy, pCLE has been studied in a wide range of indications, from peripheral nodules to lung transplantation.

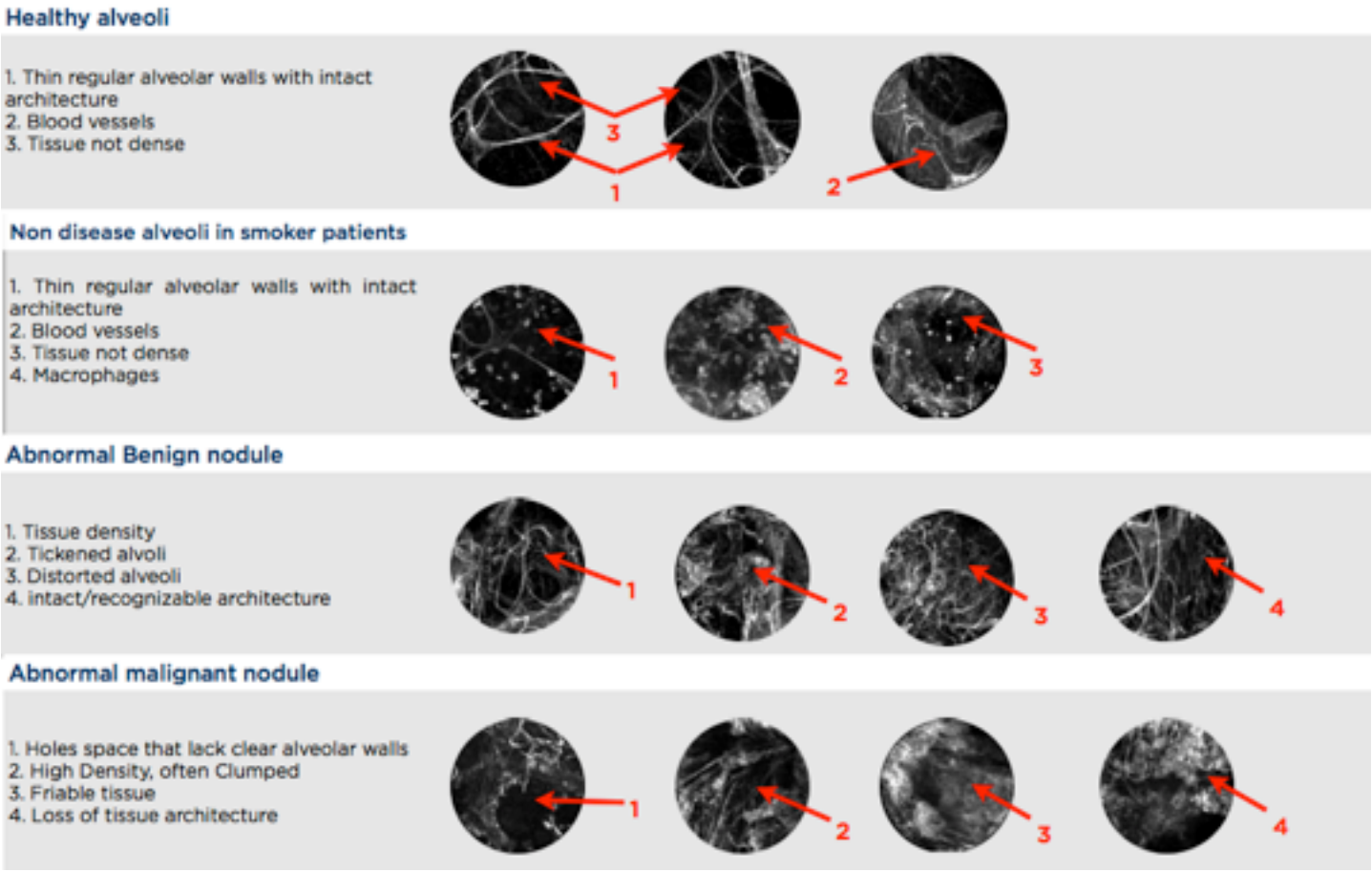

Fig.13: CLE image interpretation criteria for Respiratory Diseases (using the Alveoflex)

pCLE combined with Electromagnetic Navigational Bronchoscopy, could enhance characterization of peripheral pulmonary nodules. As described in an abstract from Arenberg et al. (61) a set of pCLE videos acquired in 29 patients in this setting were blindly reviewed to establish preliminary descriptive criteria for image interpretation and to assess the correlation of the defined criteria with either benign or malignant conditions (Figure 12). These direct comparisons of pCLE with actual histopathology have shown early promise with diagnostic sensitivity for neoplasia as high as 80%.

This preliminary classification has been recently refined, based on a retrospective review of pCLE sequences acquired on 31 pulmonary peripheral nodules suspicious for malignancy, based on clinical presentation. The pCLE images collected were categorized into three categories: normal, smokers without other diseases and abnormal. Additionally, the abnormal images were classified into non-malignant and malignant groups. Validation of these diagnostic criteria on a group of blinded investigators has shown that the pCLE criteria are highly sensitive in identifying abnormal lung tissue (100% sensitivity, 57% specificity) with a negative predictive value of 100% (positive predictive value 82%).

A prospective multicentric study is ongoing to validate these novel criteria prospectively and assess the impact of the technique on patient management.

Diffuse Peripheral Lung Diseases, and more particularly Idiopathic Interstitial Pneumonia (IIP) have been evaluated by pCLE. Over 250 patients have been explored at the Rouen University Hospital, with no severe related complication. A confocal endomicroscopy semiology based on elastin network alterations, and on fluorescent cellular infiltration has to be described, in order to interpret the distal lung confocal images .

Since pCLE allows direct observation of alveolar and vascular structures, this technique has the potential to be applied in the evaluation and assessment of lung transplanted recipients. Keller et al. (62), observed that transplant lung show active alveolitis characterized by high intra-alveolar cellularity with cells with average diameter of 24.6 + 6 µm. pCLE could potentially predict the risk of acute rejection.

pCLE could represent a new alternative in the management of critically ill patients by characterizing inflammatory infiltrates and assessing the presence of bacteria in infiltrative lung processes in acutely ill patients.

A recent study by Yserbyt et al.(63) reported the preliminary findings on the use of pCLE for the characterization of rejection after lung transplant. pCLE was used in combination with transbronchial biopsies during 105 bronchoscopies in lung transplant recipients. Autofluorescent cells were present in 73% (+10) of the recorded frames in the acute rejection (AR) group and in only 42% (+9) of the recorded frames in the control group (p=0.03).

The consecutive application of 3 pCLE criteria resulted in a sensitivity of 0.93 and a specificity of 0.83 for the detection of acute rejection. These results suggests the existence of specific pCLE characteristics in patients with AR.

Future technical developments should also enable the access and the in vivo evaluation of lymph nodes or lung nodules at a microscopic level, by inserting a confocal miniprobe in an endoscopic needle, as recently validated in pancreatic cysts. Recent and future advances in molecular imaging should also offer the potential to specifically mark and image lesions, helping to tailor patient management to specific diagnoses and findings in various lung diseases.

Summary and Future Directions

Confocal laser endomicroscopy has been shown to provide crucial and accurate real time information, making it possible to improve the diagnosis and treatment of patients with GI, pulmonary, or urology diseases.

Clinical trials have demonstrated the ability of the technique to meet unmet needs, in particular in gastroenterology indications. Where traditional tissue sampling options are limited, recent technical developments now enable real time microscopic imaging of solid organs such as pancreatic cysts, pancreatic masses, and lymph nodes. These technical developments pave the way to expanded indications to surgery, neurosurgery, gynecology, or ENT, and to optimize patient management.

This technology could also contribute to efficiency and improved patient outcomes by providing the opportunity to streamline patient management, as biomarker development could predict. Computer-based algorithms are currently being developed to help physicians shorten the learning curve in the interpretation of images, through structured training programs or real-time diagnosis support.

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