SUMMARY

This thesis attempted to answer a number of research questions. The first question was: Which digital colposcopy techniques are presently available, what is the technical background and how is their clinical efficacy? In chapter 2 we described various methods of digital colposcopy. These new techniques were all defined by the word ‘digital’ in which ‘digital’ means every method of colposcopy using any form of image enhancement by a computer. To further detail these digital colposcopy methods we discussed them under one of the following headings: digital imaging and telecolposcopy, spectroscopy, computerised colposcopy, optical coherence tomography and confocal microcolposcopy.

Photographing the cervix at colposcopy and digitalising the image was the first step on the path of development of digital colposcopy. Digital image processing techniques permit contrast enhancement of features, such as white epithelium and abnormal vascularisation, and are in this way able to help the colposcopist to identify and to grade a lesion. Digital images of the cervix can also be analysed by a computer for characteristic features and colour patterns, which may enhance the objectivity of the colposcopic examination. This process is known as computerised colposcopy and should be considered as an adjunct to conventional colposcopy, as is digital imaging. For both digital imaging processing and computerised colposcopy techniques, sensitivities and specificities above 90% are reported, but their use in day to day clinical practice is limited.

Optical coherence tomography and confocal microcolposcopy are two techniques that are least commonly used for clinical purposes within the field of gynaecology. Only one or two studies have been conducted for the use of both optical coherence tomography (a non-invasive technique that uses infrared light and provides real-time, in vivo images of the cervix with high resolution) and confocal microcolposcopy (an optical imaging technique that reconstructs 3D images with high contrast, through point illumination and a pinhole conjugate plane in front of a detector) in cervical tissue. Therefore no accurate sensitivity or specificity data can be given.
Among the most promising developments is spectroscopy, allowing a more or less automated analysis and interpretation of the colposcopic image. Spectroscopy is a non-invasive method in which for example light or electric current is used to study the biochemical composition as well as the metabolic and structural features of tissue. Digital colposcopy techniques that use spectroscopy are SpectRx, LUMATM, DySISTM, Trimodal, Truscreen® and impedance colposcopy. Sensitivity of these methods varies between 70% (Truscreen®) and 95% (SpectRx), specificity between 50% (LUMATM) and 83% (SpectRx).

Our second research question was: Are hrHPV-related lesions recognisable by colposcopy? Indeed, in chapter 3 we found that hrHPV positive lesions have other characteristics than hrHPV negative lesions. During colposcopy, the colposcopist evaluates a number of visual features of the cervix and/or lesion (i.e. mosaic and punctation patterns, vessels, acetowhitening, etc.). Visibility of the transformation zone, larger lesion size (more than 25% of the visible cervix) and coarse and irregular punctation patterns are the colposcopic features statistically significantly associated with a positive hrHPV status (OR = 2.29 (95%CI 1.41 - 3.73), OR = 1.78 (95%CI 1.08 - 2.94), and OR = 2.37 (95%CI 1.08 - 5.19) respectively).

After correction for histology (a high-grade cervical lesion is more likely to be hrHPV positive than a low-grade lesion), the difference between hrHPV positive and negative lesions for visibility of the transformation zone and lesion size remained statistically significant (OR = 2.44 (95%CI 1.35 - 4.41) and OR = 1.92 (95%CI 1.04 - 3.54) respectively). Thus these criteria, which the colposcopist already uses to form an impression of the cervix and to locate and grade a possible lesion, may help the colposcopist to identify hrHPV positive patients and as such women that are at risk for neoplastic cervical lesions.

The following research question was: Does dynamic spectral imaging colposcopy improve the sensitivity of colposcopic examination? In total 275 women were included in this Dynamic Spectral Imaging (DSI) validation study (chapter 4): 239 women were analysed in the ‘intention to treat’ (ITT) cohort and 183 women in the ‘according to protocol’ (ATP) cohort. In the ATP cohort all cases adhered strictly to the study protocol (proof of principle) and the ATP cohort is a subset of the ITT cohort. In the
ATP cohort the sensitivity of DSI colposcopy to identify patients with high-grade (CIN2+) lesions was significantly higher than of conventional colposcopy (79% (95%CI 70 - 88) and 55% (95%CI 44 - 65) respectively (p = 0.0006, asymptotic McNemar test)). When the DSI colour-coded map was combined with conventional colposcopy, the sensitivity increased further to 88% (95%CI 82 - 95). The specificity of DSI colposcopy in the ATP cohort was not significantly different from conventional colposcopy (77% (95%CI 69 - 86) and 85% (95%CI 77 - 92), respectively (p = 0.144, asymptotic McNemar test)). DSI combined with conventional colposcopy had a specificity of 69% (95%CI 60 - 78).

The data in the ITT cohort, even though not all cases were adhering to protocol criteria (e.g. not all DSI indications for high-grade lesions were sampled or the device was used even though there was a hardware problem) were analysed to approximate the performance of DSI colposcopy under clinical conditions (clinical performance). Again, in the ITT cohort the sensitivity of DSI colposcopy was significantly higher than of conventional colposcopy (65% (95%CI 56 – 74) and 52% (95%CI 42 – 61) respectively (p = 0.039, asymptotic McNemar test)). When the two techniques were combined the sensitivity was 80% (95%CI 72 - 87). The specificity of DSI colposcopy in this cohort was significantly lower than that of conventional colposcopy: 70% (95%CI 62 - 78) versus 82% (95%CI 75 - 88) (p = 0.011, asymptotic McNemar test). The combination of DSI with conventional colposcopy led to a specificity of 63% (95%CI 54 - 71) in the ITT cohort. The lower specificity means that some women are incorrectly identified as having high-grade cervical disease. However, in the clinical setting it is, in contrast to a screening setting, more important not to miss any high-grade cervical disease, which is reflected in a high sensitivity. In conclusion, both in ‘according to protocol’ and ‘intention to treat’ analysis DSI colposcopy has a significantly higher sensitivity in detecting high-grade cervical lesions than conventional colposcopy. This signifies that also in suboptimal conditions (i.e. the ITT analysis) the DSI coloscope can be a valuable asset to the colposcopic examination.

The next research questions, resulting from the previous one, were: Is the detection of cervical premalignant lesions dependent of the hrHPV type present and/or lesion size? And is there a difference between conventional colposcopy and dynamic spectral imaging (DSI) colposcopy in detection of these type-specific lesions? HrHPV type 16 positive cervical lesions have been suggested to show more defined abnormalities at colposcopy than lesions positive for other hrHPV types. So,
hrHPV type 16 positive lesions should be easier to detect at colposcopy than non type 16 hrHPV positive lesions. For that reason we studied in chapter 5 in a subset of women from the aforementioned DSI validation study the performance of DSI and conventional colposcopy in relation to the agreement between colposcopic impression and final histology. Furthermore, we explored if lesion size influences the colposcopic performance for both DSI and conventional colposcopy.

DSI defined more high-grade cervical lesions among hrHPV type 16 positive women compared to non-type 16 hrHPV positive women. This effect was seen in women with CIN2+ lesions in their final histology (p = 0.009), but also without stratifying for final histology DSI detected more high-grade cervical lesions among hrHPV type 16 positive women compared to non-type 16 hrHPV positive women (p = 0.032). Thus, in hrHPV type 16 positive women the sensitivity of DSI colposcopy for detecting CIN2+ lesions was higher than in non-type 16 hrHPV positive women (97% (95%CI 84 – 100) versus 74% (95%CI 57 – 87)). Contrastingly, this higher sensitivity in hrHPV type 16 positive women was not found for conventional colposcopy.

Moreover, we found that CIN2+ lesions defined as high-grade by DSI were significantly smaller than CIN2+ lesions defined as high-grade by the colposcopist. Since detection of lesions with conventional colposcopy occurs with the naked eye, it is likely that it is easier to miss the smaller lesions. DSI colposcopy, however, detects all high-grade cervical lesions on a per pixel level and is therefore more objective in its analysis of the cervix. This supports the hypothesis that the higher sensitivity of the DSI colposcope results from the detection of smaller high-grade lesions. One could furthermore argue that the even better sensitivity of DSI for hrHPV type 16 positive high-grade lesions is because hrHPV type 16 is a more aggressive hrHPV type causes more often high-grade abnormalities when the lesion is small in size. This is also reflected by the fact that no higher sensitivity was found for conventional colposcopy in hrHPV type 16 positive women. We could not confirm that hrHPV type 16 positive lesions have more distinct features and are therefore more easily recognised.

Women visiting the outpatient gynaecology clinic often obtain a Pap smear. Given the higher sensitivity of hrHPV-testing for detection of cervical lesions we formulated the following questions: What is the added value of hrHPV-testing to cervical cytology in a university hospital gynaecology
outpatient clinic? And is it possible to define a useful risk profile in relation to the referral reason to identify who needs beforehand testing for cervical premalignant lesions? In chapter 6 we describe our observational cohort study in which all women referred to the gynaecology outpatient clinic undergoing cervical cytology testing were co-tested for hrHPV between January and June 2007. From the Dutch nationwide network and registry of histopathology and cytopathology (PALGA) and the hospital information system all reasons for referral and follow-up data were retrieved 18 and 42 months after the last subject had entered the study. The 21 different referral reasons were categorised in three groups: women with presumed cervix pathology, women with presumed endometrial pathology and women with other referral indications (miscellaneous).

The 18 month risk for CIN2+ or CIN3+ was highest in the group with presumed cervix pathology with adjusted risks of 11.1% and 5.4% respectively. In the group with presumed endometrial pathology the 18 month risk for both CIN2+ and CIN3+ was 3.1%. The women in the miscellaneous group – women without an a priori risk for cervical or endometrial pathology - had CIN2+ and CIN3+ risks of 4.1% and 1.8% respectively. Furthermore, in this diagnostic setting and irrespective of the reason for referral, hrHPV-testing detected more CIN2/3+ lesions than cytology. This is in concordance with other studies. Therefore, for whatever reason cervical testing is performed, co-testing with hrHPV is preferred. The added value of using both hrHPV-testing and cytology lies mainly in detecting lesions in women with normal cytology, who otherwise would not be detected. Furthermore, it seems reasonable not to routinely take smears as part of the intake procedure at a gynaecologic outpatient clinic, except for those women referred for presumed cervical and endometrial pathology.

Although screening with hrHPV as an alternative for cervical cytology has many advantages (i.e. higher sensitivity, the possibility to perform the test in self-sampled material, etc.), sole hrHPV-testing has a high false positive rate. The reason for this is that a transient hrHPV infection that will not cause any premalignant cervical lesions cannot be distinguished from a persistent hrHPV infection that will develop into a premalignant lesion in need of treatment. Therefore, additional triage markers are necessary to identify those women that are at risk, for example cytology combined with hrHPV genotyping, p16/Ki-67 dual-stained cytology or methylation markers. We focussed on methylation markers: single CADM1 and single MAL promoter methylation analysis has been researched, but
without satisfactory results for use in a clinical population. This led to the formulation of the last research question: Is it possible to develop a panel of clinically useful methylation markers to identify hrHPV positive women at risk for high-grade cervical lesions and cervical cancer?

In abovementioned study (chapter 7) combined methylation analysis for CADM1 and MAL was tested as a triage marker for hrHPV positive women, instead of using methylation analysis for just CADM1 or just MAL. Two quantitative methylation-specific PCR’s (qMSP) for CADM1 (regions M12 and M18) and two qMSP’s for MAL (regions M1 and M2) were applied to 261 cervical tissue specimens, ranging from healthy tissue to cervical carcinoma. Most CIN3+ lesions were detected if combining two qMSPs representing both CADM1 and MAL: up to 99% of the carcinomas were positive. Further analysis of hrHPV positive smears led to the conclusion that a combination of CADM1-M18 and MAL-M1 had the best sensitivity and specificity to detect high-grade cervical lesions and cervical cancer. This panel of methylation markers performed at least as good as cytology in terms of sensitivity and specificity.

The final step in this study was to apply these data to a clinical population. Therefore CADM1-M18 and MAL-M1 methylation analysis was applied to 79 hrHPV positive women visiting an outpatient colposcopy clinic because of abnormal cytology. HrHPV-testing had a sensitivity of 97% and a specificity of 33% for CIN3+ lesions in this population. Adding CADM1-M18 / MAL-M1 methylation analysis increased the specificity to 78% with a sensitivity of 70%. So to conclude, in this study the CADM1-M18 / MAL-M1 methylation panel was at least as good as cytology. Therefore, combination of CADM1 and MAL methylation analysis may be useful as an alternative molecular triage tool for hrHPV positive women. In this way, women can be further stratified into risk profiles for cervical pathology, making the selection for the clinician easier who to refer for colposcopy, although the sensitivity and specificity of this methylation panel should be further improved.