INNOVATION

Optical imaging for cervical cancer detection: solutions for a continuing global problem

Nadhi Thekkek and Rebecca Richards-Kortum

Abstract | Cervical cancer is the leading cause of cancer death for women in developing countries. Optical technologies can improve the accuracy and availability of cervical cancer screening. For example, battery-powered digital cameras can obtain multi-spectral images of the entire cervix, highlighting suspicious areas, and high-resolution optical technologies can further interrogate such areas, providing *in vivo* diagnosis with high sensitivity and specificity. In addition, targeted contrast agents can highlight changes in biomarkers of cervical neoplasia. Such advances should provide a much needed global approach to cervical cancer prevention.

Cervical cancer is the second most common cancer in women worldwide[1]; more than 80% of cervical cancers occur in the developing world where the fewest resources exist for management[1]. Most cases of cervical cancer can be prevented through screening programmes, such as the Papanicolaou (Pap) smear, which is aimed at detecting precancerous lesions for treatment. In countries in which organized programmes have been established, the incidence and mortality of cervical cancer have dramatically reduced[2]. However, the necessary resources and infrastructure for screening are not available in many countries and, as a result, 274,000 women die each year from this preventable disease[1].

Cervical cancer is caused by chronic infection with high-risk types of human papillomavirus (HPV)[3]. The recent development of vaccines to prevent HPV infection promises to further reduce the incidence of cervical cancer in countries where vaccines are available[4]. However, the significant cost of vaccines and, in some cases, political or logistical barriers could delay implementation of universal mass vaccination in many developing countries[5]. Furthermore, current vaccines are effective only against high-risk HPV types 16 and 18, which together account for approximately 70% of cervical cancers worldwide[5]. Because the vaccine does not cover all high-risk HPV subtypes, routine cervical cancer screening is necessary even for women who have been vaccinated and will remain so for the foreseeable future.

Clinical approaches to screening

Current screening and diagnosis of cervical precancer is based on optical techniques developed in the early 1900s[2]. As shown in the top row of FIG. 1, an abnormal Pap smear is followed by examination of the cervix using a low-power light microscope (colposcope) to visualize changes in tissue reflectance, which might indicate the presence of a precancerous lesion. For decades, clinical investigators have searched for ways to improve the optical contrast between normal cervical tissue and a precancerous lesion. The use of simple agents such as acetic acid and Lugol's iodine, together with a green illumination filter, can highlight suspicious regions. However, because the specificity of visual examination is low, colposcopically abnormal areas are routinely biopsied to confirm the presence of disease[6]. Implementing this approach requires extensive infrastructure, personnel and economic resources; as a result, the vast majority of women in the world do not have access to life-saving screening programmes[7].

Simple visual approaches have been explored to enable cervical cancer screening in resource-poor settings. For example, the use of visual inspection with acetic acid (VIA) is being explored as an alternative to Pap smear screening and colposcopy in many developing countries[7–10]. In VIA, a trained health-care provider examines the cervix with the naked eye before and after application of acetic acid to look for acetowhitening of the tissue. VIA has many advantages: it is inexpensive, requires minimal infrastructure and if abnormal areas are observed the patient can be referred for immediate treatment, circumventing the need for histological samples and the attendant cost. However, because VIA relies on subjective visual interpretation, it is crucial to define consistent criteria for suspicious lesions and to train providers to correctly implement these criteria. Large clinical trials have been conducted to evaluate the performance of VIA for screening; TABLE 1 summarizes the findings of several of these trials. A recent review of the performance of VIA in six studies involving more than 65,000 women in South Africa, India, Zimbabwe, China, Burkina Faso, Congo, Guinea, Mali and Niger found that the sensitivity of VIA varied from 67% to 79% and specificity ranged from 49% to 86%. The sensitivity of VIA is similar to that reported for Pap smear screening but specificity is lower, although some studies suffered from verification bias[11]. The use of low-level magnification does not improve the performance of VIA appreciably[12,13]. Denny noted that restricting the definition of a positive VIA test to a well-defined acetowhite lesion significantly improved specificity but reduced sensitivity[13]. In a series of 1,921 women screened in Peru, Jeronimo found that the VIA positivity rate dropped from 13.5% in the first few months to 4% during subsequent months of a 2-year study; the drop was hypothesized to be due to a learning curve for the evaluator[10].

Recent advances in consumer electronics have led to inexpensive, high-dynamic-range charge-coupled device cameras with excellent low-light sensitivity. These technologies have been used to acquire digital images of the cervix in a relatively inexpensive way, with or without magnification[14]. Moreover, automated image diagnosis algorithms based on modern image processing techniques can assist and complement subjective visual interpretation[15–17]. These approaches, which we refer to as digital inspection with acetic acid (DIA), can potentially improve the performance of VIA.

Changes in optical properties

Both VIA and colposcopy rely on changes in the optical properties of neoplastic tissue to detect precancerous lesions. Image contrast between normal tissue and precancerous areas can be enhanced in a number of ways. Illuminating tissue with green light during colposcopy highlights the contrast associated with atypical blood vessels because haemoglobin absorbs green light.

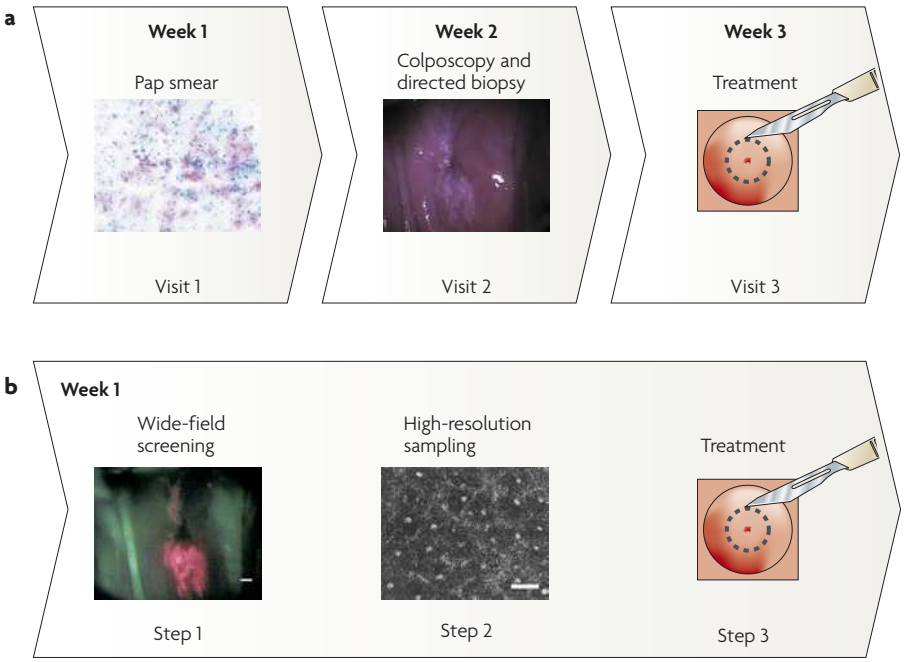

Figure 1 | **Current and future cervical screening techniques. a** | Currently, screening, detection and treatment of cervical cancer and its precursors is labour- and resource-intensive. The entire process requires up to three visits over the course of a few weeks. Following the initial screening exam at the first visit, the patient and the primary health-care provider must wait 1–2 weeks for Papanicolaou (Pap) smear results. If positive, a second visit must be scheduled to perform colposcopy and directed biopsy in order to confirm a diagnosis of precancer. Biopsy results are typically not available for 1-2 weeks. If a biopsy shows high-grade precancer, treatment can be scheduled at a third visit. Although this strategy has decreased the incidence of cervical cancer in many developed countries, the infrastructure and resources necessary for comprehensive screening are unavailable in many developing countries. **b** | Optical technologies that combine wide-field imaging for high sensitivity with high-resolution interrogation for high specificity can streamline this procedure and provide immediate diagnosis at the point of care, potentially enabling diagnosis and treatment in one clinic visit. Low-cost optical devices may eventually allow more women in developing countries to have access to screening. At the same time, they may make detection and treatment more accessible and convenient to women in all regions of the world. Scale bars in the colposcopy images are 2 mm, and the scale bar in the confocal image is 50 µm. Images reproduced, with permission, from REF. 39 © Elsevier Inc. (2005) and REF. 61 © Elsevier Inc. (2002).

The application of acetic acid differentially increases the light scattering of neoplastic lesions[18], making them easier to visualize. Although these simple approaches help clinicians recognize suspicious lesions, they exploit only a few of the changes in optical properties that are associated with the development of neoplasia. Recent studies have characterized a broad range of changes in tissue optical properties that occur with precancerous changes[19–21]. Results of these studies are summarized in FIG. 2 and indicate that optical methods can be used to probe known hallmarks of cancerous changes in tissue, such as epithelial cell morphology, metabolic activity and differentiation[22], stromal angiogenesis[23,24] and epithelial–stromal communication[25].

Light scattering and absorption. Optical technologies can interrogate changes in tissue architecture, cell morphology and biochemical composition. Most high-grade precancers present with vascular changes due to the development of new blood vessels[26]. This angiogenesis can be visualized, and can be quantified using image-analysis approaches[17]. Haemoglobin has a characteristic absorption spectrum with peaks at 420 nm, 542 nm and 577 nm. Changing the wavelength of illumination can enhance vascular contrast and can probe vessels at different depths below the visual surface of the cervix. Acetic acid increases light scattering from cervical cell nuclei. Following application of acetic acid[18,27], the mean scattering coefficient of precancerous tissue is approximately three times higher than that of normal epithelium[28,29]. The difference in scattering between normal and precancerous epithelium is attributed to increased nuclear size, increased optical density of the nucleus and changes in chromatin texture[30,31] that have been documented in cancerous cells. Finally, cervical precancer is associated with decreased stromal scattering, attributed to a degradation of collagen fibres that is possibly due to proteases secreted by pre-neoplastic epithelial cells[32].

Autofluorescence. The use of fluorescence interrogation can extend the range of biochemical changes that can be probed optically. In normal cervical tissue, collagen crosslinks give rise to bright fluorescence in the stroma over a broad range of excitation wavelengths[20,33]. In women with normal cervical tissue, this stromal fluorescence increases with age and menopause[21]; however, stromal fluorescence is greatly diminished in cervical precancers[20,33] and cancers[34]. Ultraviolet (UV) excitation wavelengths (~330–370 nm) and green excitation wavelengths (~510–550 nm) have been used to monitor autofluorescence in cervical epithelial cells. Cytoplasmic autofluorescence seen on exposure to UV excitation wavelengths has been attributed to mitochondrial NADH; similarly mitochondrial FAD fluoresces on exposure to green excitation wavelengths[20,33]. In addition, cervical epithelial cells show autofluorescence at the cell periphery, which is often attributed to cytokeratins[28]. In normal epithelium, basal epithelial cells show strong cytoplasmic fluorescence and parabasal, intermediate and superficial cells show fluorescence only at the periphery of the cell[20,33]. All of this information can be exploited to determine the presence of precancerous lesions. FIGURE 2 compares confocal fluorescence images of organ cultures of normal human cervical tissue and precancerous tissue. In low-grade precancers, cytoplasmic fluorescence is visible in the bottom third of the epithelium and in high-grade precancers cytoplasmic fluorescence is visible throughout the lower two-thirds of the epithelium, with reduced fluorescence attributed to keratin[20,33]. This is consistent with recent studies which show that HPV-immortalized keratinocytes show increased NADH and FAD fluorescence relative to normal keratinocytes[35]. However, to take advantage of this knowledge for the benefit of the patient, new imaging technologies need to be developed.

Table 1 | **Recent investigations of *in vivo* optical imaging techniques for early detection**

Type of detection	Patients (*n*)	Sensitivity (%)/ specificity (%)	Type of study	Refs
Major trials				
Colposcopy	5,378	85/69	Prospective	6
VIA	2,148	77/64	Prospective	89
	2,817	67/83*	Prospective	90
	1,997	71/74*	Prospective	91
	2,575	70/79	Prospective	13
	1,093	79/49	Prospective	92
	54,981	79/86	Prospective	93
Wide-field	604	92/50	Prospective	44
	111	97/70*	Prospective	46
	572	95/55	Prospective	47
Pilot studies				
Spectroscopy	161	83/80	Cross-validation	51
	44	92/90	Cross-validation	52
High-resolution‡	28§	100/100	Prospective	29
	38§	100/91	Retrospective	66

*The threshold for positive test result is low-grade dysplasia. For all others the threshold is high-grade dysplasia. ‡Studies carried out in *ex vivo* tissue. §*n* is the number of biopsies. VIA, visual inspection with acetic acid.

In vivo optical imaging technologies

In the past decade, advances in high-performance, low-cost electronics have enabled development of sensitive systems for optical imaging and interrogation of cervical precancer *in vivo*[36–38]. TABLE 2 summarizes a variety of different optical interrogation methods currently under investigation. As illustrated in the bottom row of FIG. 1, these optical tools can be used to monitor biologically predictive features of cervical cancer, providing a global approach to detect cervical cancer that bridges the molecular, cellular and tissue scales.

Multispectral wide-field imaging. Wide-field imaging relies on cameras to image changes in reflectance and autofluorescence at multiple wavelengths across the entire cervical epithelium. Typically, wide-field imaging can achieve 50–100 μm spatial resolution and can highlight suspicious regions of tissue. Point-probe techniques, which use a small fibre-optic probe placed in contact with the tissue surface, can then be used to interrogate suspicious areas with higher spatial or spectral resolution.

A number of pilot studies have investigated the features of multispectral reflectance imaging to assess which give the greatest image contrast. Reflectance imaging with green wavelength illumination consistently gives the best contrast because of haemoglobin absorption[39]. Alternatively, colour reflectance images obtained with white light illumination can be separated into red, green and blue channels and analysed to enhance image contrast[15,40]. A pilot study of digital reflectance images of the cervix acquired from 29 women showed that an automated image analysis algorithm could identify the presence and spatial extent of high-grade precancers with 79% sensitivity and 88% specificity compared with histopathological analysis[15]. Illuminating tissue with light that has been passed through a linear polarizer and imaging reflectance through an orthogonally oriented linear polarizer can reduce specular reflection — bright areas caused by light reflected from the tissue surface — and improve visualization of subepithelial vascular patterns[39].

Imaging the time course of aceto-whitening can also improve the ability to discriminate high-grade precancer[39,40]; a multispectral reflectance imaging study of 123 women found that the increase in light scattering after application of acetic acid was greater and persisted for a longer time in high-grade precancers[41].

Similarly, tissue autofluorescence can be imaged in wide-field mode[42]. Exciting autofluorescence in the UV and blue wavelengths (~440–470 nm) has been shown to give the greatest contrast between normal and precancerous tissue[42,43]. Results of several large clinical studies investigating hyperspectral autofluorescence and reflectance imaging have recently been reported[44–47] and are summarized in TABLE 1. In these studies, autofluorescence and reflectance spectral data were collected with relatively high spectral (~5 nm) and spatial (~1 mm) resolution. Pattern recognition approaches were used to classify tissue and results were compared with the gold standard of histology to assess performance. In a study of 111 women, Ferris found a sensitivity of 97% and a specificity of 70% for hyperspectral wide-field imaging compared with colposcopy-directed biopsy or loop electrosurgical excision[46]. A larger series of 572 women were assessed with this device[47], yielding a sensitivity of 95.1% and a specificity of 55.2%. Huh and colleagues found similar results using a different hyperspectral imaging approach to detect UV-induced fluorescence (at 337 nm) and reflectance in 604 women[44]. With a sensitivity of 92% and a specificity of 50%, they found that hyperspectral imaging could detect one-third more high-grade precancers than colposcopy alone, with a relatively small increase in the false-positive rate[44]. In a multicentre trial testing the device as an adjunct to colposcopy in 193 women, researchers found that the use of hyperspectral imaging resulted in a 22% relative gain in the true-positive rate of colposcopy with an 18.1% incremental gain in the false-positive rate[48]. A multicentre trial of this device involving 2,299 women randomized to receive colposcopy alone or colposcopy plus hyperspectral imaging showed similar results[45]. In addition, for women with a Pap smear showing atypical cells of uncertain significance or low-grade precancer, hyperspectral imaging increased the true-positive rate by 26.8% compared with colposcopy alone, with a minimal increase in the false-positive rate.

This device was approved by the US Food and Drug Administration in March of 2006 to enhance the sensitivity of colposcopic detection of high-grade cervical precancers[49]. Thus, although wide-field imaging can objectively detect cervical precancers with high sensitivity, specificity is limited. The use of other optical approaches to probe suspicious areas identified with wide-field imaging may increase specificity. Two approaches that have been considered are point optical spectroscopy and high-resolution optical imaging.

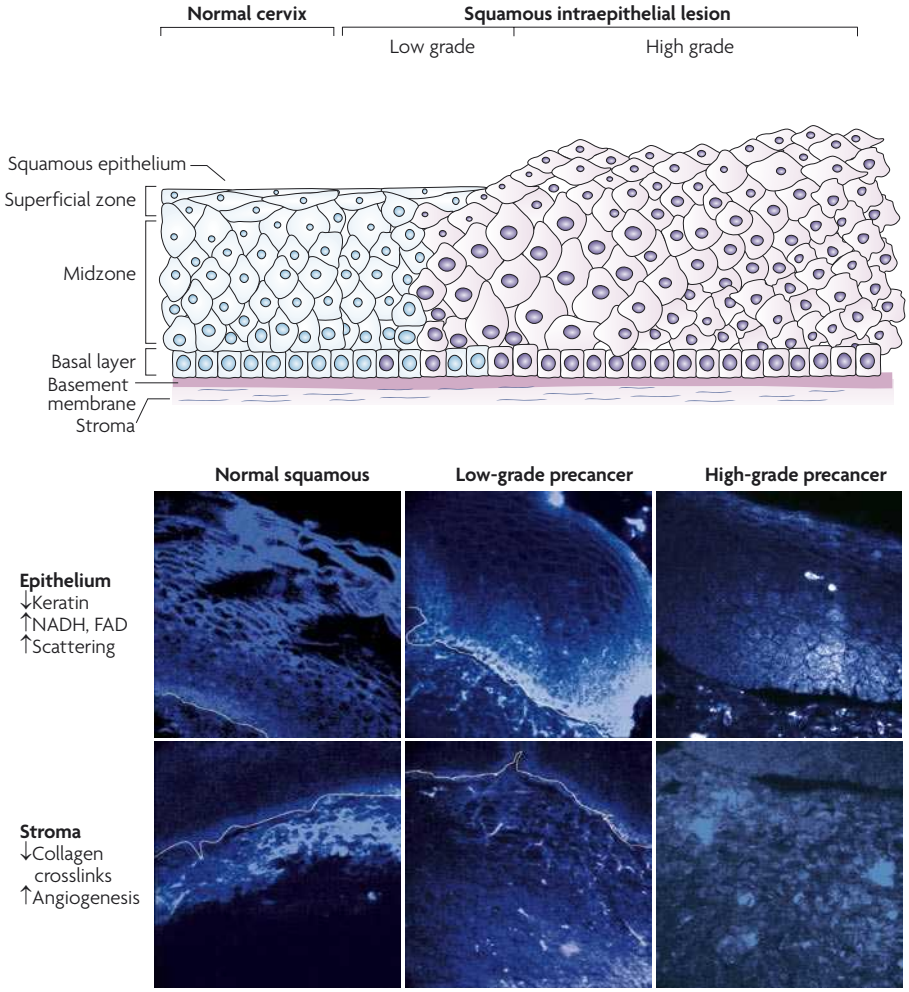

Figure 2 | **The development of cervical precancer leads to changes in the optical properties of both the epithelium and the stroma.** As precancer develops, stromal angiogenesis leads to increased stromal absorption. Precancerous epithelial cells show increased light scattering, which can be further enhanced with addition of acetic acid. In normal cervical tissue, collagen crosslinks within the stroma are strongly fluorescent; this fluorescence decreases with the development of precancer. In the normal squamous epithelium, basal epithelial cells show cytoplasmic fluorescence associated with mitochondrial NADH and FAD whereas superficial epithelial cells show peripheral fluorescence attributed to keratin. With precancer development, keratin fluorescence decreases in the epithelium and mitochondrial fluorescence is found in cells throughout progressively more superficial regions of the epithelium. These biologically predictive features of cervical precancer can be measured using optical technologies at the point of care. Images reproduced, with permission, from REF. 28 © Blackwell Publishing (2003).

Optical spectroscopy. Fibre-optic probes can be used to record fluorescence and reflectance spectra of small areas of tissue with 1–5 nm spectral resolution, providing detailed, quantitative information about the distributions of optically active molecules within a tissue (FIG. 3).

A number of studies have been carried out to assess whether optical spectroscopy can provide accurate *in vivo* diagnosis of cervical precancer; TABLE 1 summarizes results of key studies. Reflectance spectroscopy measures the intensity of light reflected as a function of illumination wavelength, providing information about changes in epithelial cell scattering, stromal scattering and stromal angiogenesis. Using empirical algorithms to classify tissue on the basis of reflectance alone achieved sensitivity of 72% and specificity of 81% in one series of 161 patients[50], but approaches that use fluorescence spectroscopy alone or the combination of reflectance and fluorescence generally yield better classification accuracy[51,52]. Much current effort is focused on the design of fibre-optic probes[53,54] and on analysis strategies to separate reflectance signals from the epithelium and the stroma[32,55] in an attempt to increase accuracy.

In vivo fluorescence spectroscopy can detect high-grade precancer with good accuracy. Early pilot studies focused on UV and blue excitation wavelengths[56,57]. More recently, a study of 146 patients comparing 18 different excitation wavelengths found that three broad ranges of excitation — 330–340 nm (UV), 350–380 nm (UV) and 400–450 nm (blue) — gave the best sensitivity and specificity for detection of high-grade precancer[58]. Across all studies, the fluorescence intensity of precancerous lesions is lower than that of normal squamous tissue, and the peak emission wavelength of precancers is shifted to longer emission wavelengths relative to that of normal tissue[56–59]. The decreased fluorescence intensity has been attributed to the decreased fluorescence and increased absorption in the stroma in cervical precancers[60–62], whereas the spectral shift is attributed to both increased haemoglobin absorption and increased mitochondrial fluorescence in precancers[60–62]. Drezek showed that at 380 nm excitation approximately 20% of detected fluorescence of squamous normal tissue is due to NADH, whereas 40–50% of detected fluorescence in high-grade precancer is due to NADH[60]. Brookner found that the fluorescence of columnar normal tissue and metaplasia are lower than that of squamous normal tissue[63]. The performance of optical algorithms is often limited by the challenge of discriminating precancers at the junction between squamous and columnar epithelium, where cervical precancers frequently develop.

Several small studies have compared the performance of reflectance and fluorescence spectroscopy alone and in combination with reflectance spectroscopy for cervical precancer detection[51,52,64], with combined methods giving the best results. Georgakoudi discovered that combining three modes of spectroscopy — fluorescence, reflectance and light scattering — yielded better results than any individual mode[52]. Mirabal noted that reflectance spectroscopy could distinguish columnar normal epithelium from high-grade dysplasia with higher specificity than fluorescence alone[50].

Importantly, Weingandt noted that inflammatory lesions can give rise to false-positive fluorescence measurements[59], and recent studies in other organ sites indicate that inflammation and precancer both exhibit a similar loss in stromal autofluorescence[65]. Techniques that better

Table 2 | **Overview of optical technologies being used for the detection of cervical cancer**

Technique	Spatial resolution	Field of view	Depth	Sources of contrast	Cost	In clinical use?
Visual inspection	100–200 μm	Entire cervix	Surface	Induced change in scattering	$	Yes
Wide-field imaging	50–100 μm	Entire cervix	Surface	Fluorescence: collagen Reflectance: haemaglobin absorption, acetic acid-induced change in scattering	$$–$$$$	Yes
Spectroscopy	1 mm	1 mm	0.3–1 mm	Fluorescence: collagen, NADH, FAD Reflectance: haemaglobin absorption, morphological changes, DNA content, chromatin texture	$$–$$$	No
High-resolution imaging*	1–2 μm	<1 mm	<1 mm	Fluorescence: fluorescently labelled probes Reflectance: acetic acid-induced change in scattering, morphological changes, scattering coefficient	$$$$	No

*Laser scanning confocal microscopy. $, <$100; $$, $100–$5,000; $$$, $5,000–$30,000; $$$$, >$30,000 (in US dollars).

probe changes in epithelial signatures, such as depth-resolved spectroscopy[54] or high-resolution imaging[29,66], may give rise to better specificity.

High-resolution imaging. Small, flexible confocal microscopes have been developed to image cervical tissue and microfabrication techniques can be used to manufacture confocal microscopes with minimal power requirements. High-resolution techniques can image tissue with sub-cellular resolution to probe changes in epithelial cell morphology and epithelial architecture without the need for biopsy, sectioning and staining[67] (FIG. 3). Video-rate reflectance confocal microscopy yields images of intact epithelial tissue with 1–2 μm spatial resolution[68] and, with the use of acetic acid, determination of image parameters such as the nuclear to cytoplasmic (N:C) ratio are possible[69]. Collier showed that the N:C ratio measured by confocal microscopy could separate high-grade cervical precancers with a sensitivity and specificity greater than 90%[29,66]. Automated image analysis routines can be used to segment nuclei in confocal images of cervical tissue and objectively calculate the N:C ratio[70]. More recently, fibre-optic confocal microscopes have become available to acquire confocal images of cervical tissue *in vivo* at near video rate in both reflectance[71] and fluorescence modes[72]. Although it is difficult to image weak autofluorescence *in vivo* using confocal fluorescence microscopy owing to photobleaching limits, advances in optically active, targeted contrast agents can be used to tag biomarkers of interest with an optical signal that can be measured and quantified *in vivo*.

Contrast agents for molecular imaging

Recent developments in confocal fluorescence imaging have shown the utility of new vital stains, such as intravenously administered fluorescein, in highlighting vascular changes and topically applied acriflavine to visualize cell nuclei[73]. In the past decade, enormous progress has been made in understanding the molecular events that accompany carcinogenesis. Optically active, molecular-targeted contrast agents can be used to image these biomarkers *in vivo*[74,75].

In general, targeted optical contrast agents consist of a probe molecule, such as an antibody[76] or peptide[77], conjugated to an optically interrogatable label[74], such as metal nanoparticles[78,79], quantum dots[80] and organic fluorescent dyes[76]. Fluorescent dyes conjugated to monoclonal antibodies can target multiple cell surface receptors that are overexpressed on tumour cells, such as the epidermal growth factor receptor (EGFR)[76]. Alternatively, peptides such as EGFR can be used to bind to specific receptors[77]. These agents have a smaller molecular weight, giving them the additonal advantage that they can be used topically. The broad excitation range and narrow emission spectra of quantum dots provides the ability to simultaneously image expression of multiple biomarkers[81,82], although concerns exist about the cytotoxicity of these materials[83].

As an alternative, some contrast agents incorporate optically active metal nanoparticles[74,78,79,82]. Gold and silver nanoparticles provide a strong source of backscattered light for contrast in wide-field and high-resolution imaging[84]; the scattering signal from a single nanoparticle has been shown to be equivalent to approximately 1 million fluorophores[85]. Unlike fluorescent dyes, metal nanoparticles are not susceptible to photobleaching, and gold is non-toxic and biocompatible[79]. Gold nanoparticles conjugated to anti-EGFR antibodies have been used to image cervical precancer *in vitro* with high contrast[78,79]. EGFR-overexpressing cells induce aggregation of gold nanoparticles, leading to non-linear enhancements in scattering that can magnify signal differences resulting from moderate levels of overexpression[78]. Indeed, in one study of normal cervical tissue and high-grade precancerous tissue labelled with anti-EGFR gold nanoparticles, this aggregation increased the image-to-contrast ratio 10–20-fold, well beyond values reported for antibody-targeted fluorescent dyes[78].

Conclusions and perspectives

New screening technologies should work for developed and developing countries. The decreasing incidence of cervical cancer in many developed countries is a testament to the impact of comprehensive screening programmes[86]. As current HPV vaccines do not prevent all cervical cancers and women in low-resource areas may not have access to new vaccines for decades, we must continue developing low-cost, high-impact screening technologies that can reduce the incidence of cervical cancer worldwide.

Optical imaging and spectroscopy can non-invasively assess the morphological and biochemical changes associated with the development of precancer at the point of care. Driven by advances in consumer electronics, high-quality optical images can now be obtained with low-cost devices; tandem advances in digital signal processing provide

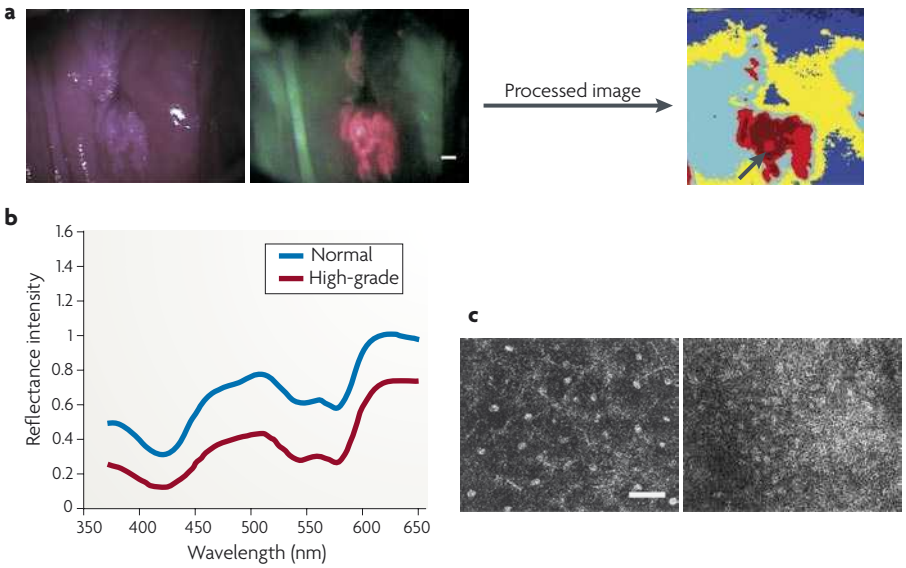

Figure 3 | **Wide-field imaging and spectroscopy. a** | Wide-field reflectance and autofluorescence imaging can interrogate the entire field of the cervix, indicating suspicious areas; digital image analysis approaches can help to objectify and automate the recognition of abnormal areas with high sensitivity. High spectral or spatial resolution techniques can be used to probe suspicious areas (arrow) to confirm diagnosis of precancerous areas. Spectroscopy (**b**) can probe changes in the concentration of tissue chromophores, whereas confocal microscopy (**c**) can directly image changes in cell morphology and nuclear to cytoplasmic ratio without the need to biopsy, section and stain tissue. Scale bars measure 2 mm in the wide-field images, and 50 μm in the confocal images. Part **a** is reproduced, with permission, from REF. 39 © Elsevier Inc. (2005). Part **b** is reproduced, with permission, from REF. 26 © Society of Photo-Optical Instrumentation Engineers (2006). Part **c** is reproduced, with permission, from REF. 61 © Elsevier Inc. (2002).

the ability to automate image analysis. Thus, optical imaging is ideally suited for use as a screening technology. Results of large, multicentre trials of wide-field hyperspectral imaging show that this approach has high sensitivity but lower specificity. In addition, currently available imaging instrumentation is expensive and bulky, making it difficult to use in low-resource settings[87]; efforts to engineer lower-cost, battery-powered, portable devices are essential to support global translation. Further work is needed to improve specificity, and efforts should focus on improving the ability to discriminate precancer from normal columnar tissue, metaplasia and inflammation.

Alternatively, depth-resolved spectroscopy or high-resolution optical imaging may provide complementary information about optical changes in the epithelium, which can improve specificity. In particular, the sensitivity and specificity of high-resolution *in vivo* imaging in pilot studies, coupled with recent developments in low-cost, high-resolution fluorescence imaging systems[88] makes this approach especially appealing. However, large-scale clinical trials are needed to confirm and optimize diagnostic performance of high-resolution approaches. Harnessing the benefits of optically active, targeted contrast agents to image cancer-related biomarkers may further aid performance.

As shown in FIG. 3, optical technologies provide a flexible approach to sample the full range of biochemical and morphological changes that accompany the development of precancerous lesions. This multi-modal optical approach has the potential to improve the performance of precancer screening and, once appropriately validated, also has the potential to expand access to screening.

Nadhi Thekkek and Rebecca Richards-Kortum are at the Department of Bioengineering, Rice University, 6,100 Main Street, Houston, Texas 77005, USA.
Correspondence to R.R-K.
e-mail: rkortum@rice.edu

doi:10.1038/nrc2462
Published online 14 August 2008

1. Parkin, D. M., Bray, F., Ferlay, J. & Pisani, P. Global cancer statistics, 2002. *CA Cancer J. Clin.* **55**, 74–108 (2005).
2. Koss, L. G. The Papanicolaou test for cervical cancer detection. A triumph and a tragedy. *JAMA* **261**, 737–743 (1989).
3. Walboomers, J. M. *et al.* Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J. Pathol.* **189**, 12–19 (1999).
4. Cohen, J. Public health. High hopes and dilemmas for a cervical cancer vaccine. *Science* **308**, 618–621 (2005).
5. Agosti, J. M. & Goldie, S. J. Introducing HPV vaccine in developing countries — key challenges and issues. *N. Engl. J. Med.* **356**, 1908–1910 (2007).
6. Mitchell, M. F., Schottenfeld, D., Tortolero-Luna, G., Cantor, S. B. & Richards-Kortum, R. Colposcopy for the diagnosis of squamous intraepithelial lesions: a meta-analysis. *Obstet. Gynecol.* **91**, 626–631 (1998).
7. Goldie, S. J. *et al.* Cost-effectiveness of cervical-cancer screening in five developing countries. *N. Engl. J. Med.* **353**, 2158–2168 (2005).
8. Sankaranarayanan, R. *et al.* Visual inspection of the uterine cervix after the application of acetic acid in the detection of cervical carcinoma and its precursors. *Cancer* **83**, 2150–2156 (1998).
9. Sankaranarayanan, R. *et al.* Effect of visual screening on cervical cancer incidence and mortality in Tamil Nadu, India: a cluster-randomised trial. *Lancet* **370**, 398–406 (2007).
10. Jeronimo, J. *et al.* Visual inspection with acetic acid for cervical cancer screening outside of low-resource settings. *Rev. Panam. Salud Publica* **17**, 1–5 (2005).
11. Sankaranarayanan, R., Gaffikin, L., Jacob, M., Sellors, J. & Robles, S. A critical assessment of screening methods for cervical neoplasia. *Int. J. Gynecol. Obstet.* **89** (Suppl. 2), S4–S12 (2005).
12. Sankaranarayanan, R. *et al.* The role of low-level magnification in visual inspection with acetic acid for the early detection of cervical neoplasia. *Cancer Detect Prev.* **28**, 345–351 (2004).
13. Denny, L., Kuhn, L., Pollack, A. & Wright, T. C. Jr. Direct visual inspection for cervical cancer screening: an analysis of factors influencing test performance. *Cancer* **94**, 1699–1707 (2002).
14. Thekkek, N., Martinez, J., Follen, M. & Richards-Kortum, R. Digital imaging aid for the early detection of cervical cancer in low resource settings. *Biomed. Eng. Soc. 2007 Annu. Fall Meet.* (Los Angeles, California, 2007).
15. Park, S. Y. *et al.* Automated image analysis of digital colposcopy for the detection of cervical intraepithelial neoplasia. *J. Biomed. Opt.* **13**, 014029 (2008).
16. Cristoforoni, P. M. *et al.* Computerized colposcopy: results of a pilot study and analysis of its clinical relevance. *Obstet. Gynecol.* **85**, 1011–1016 (1995).
17. Mikhail, M. S., Palan, P. R., Basu, J. & Romney, S. L. Computerized measurement of intercapillary distance using image analysis in women with cervical intraepithelial neoplasia: correlation with severity. *Acta Obstet. Gynecol. Scand.* **83**, 308–310 (2004).
18. Drezek, R. A. *et al.* Laser scanning confocal microscopy of cervical tissue before and after application of acetic acid. *Amer. J. Obstet. Gynecol.* **182**, 1135–1139 (2000).
19. Skala, M. C. *et al.* Multiphoton microscopy of endogenous fluorescence differentiates normal, precancerous, and cancerous squamous epithelial tissues. *Cancer Res.* **65**, 1180–1186 (2005).
20. Pavlova, I. *et al.* Microanatomical and biochemical origins of normal and precancerous cervical autofluorescence using laser-scanning fluorescence confocal microscopy. *Photochem. Photobiol.* **77**, 550–555 (2003).
21. Brookner, C. K. *et al.* Autofluorescence patterns in short-term cultures of normal cervical tissue. *Photochem. Photobiol.* **71**, 730–736 (2000).
22. Gulledge, C. J. & Dewhirst, M. W. Tumor oxygenation: a matter of supply and demand. *Anticancer Res.* **16**, 741–749 (1996).
23. Smith-McCune, K. K. & Weidner, N. Demonstration and characterization of the angiogenic properties of cervical dysplasia. *Cancer Res.* **54**, 800–804 (1994).
24. Guidi, A. J. *et al.* Vascular permeability factor (vascular endothelial growth factor) expression and angiogenesis in cervical neoplasia. *J. Natl Cancer Inst.* **87**, 1237–1245 (1995).
25. Davidson, B. *et al.* Expression of matrix metalloproteinase-9 in squamous cell carcinoma of the uterine cervix-clinicopathologic study using immunohistochemistry and mRNA *in situ* hybridization. *Gynecol. Oncol.* **72**, 380–386 (1999).
26. Triratanachat, S., Niruthisard, S., Trivijitsilp, P., Tresukosol, D. & Jarurak, N. Angiogenesis in cervical intraepithelial neoplasia and early-staged uterine cervical squamous cell carcinoma: clinical significance. *Int. J. Gynecol. Cancer* **16**, 575–580 (2006).
27. Zuluaga, A. F. *et al.* Contrast agents for confocal microscopy: how simple chemicals affect confocal images of normal and cancer cells in suspension. *J. Biomed. Opt.* **7**, 398–403 (2002).
28. Collier, T., Follen, M., Malpica, A. & Richards-Kortum, R. Sources of scattering in cervical tissue: determination of the scattering coefficient by confocal microscopy. *Appl. Opt.* **44**, 2072–2081 (2005).

29. Collier, T., Guillaud, M., Follen, M., Malpica, A. & Richards-Kortum, R. Real-time reflectance confocal microscopy: comparison of two-dimensional images and three-dimensional image stacks for detection of cervical precancer. *J. Biomed. Opt.* **12**, 024021 (2007).
30. Drezek, R. *et al.* Light scattering from cervical cells throughout neoplastic progression: influence of nuclear morphology, DNA content, and chromatin texture. *J. Biomed. Opt.* **8**, 7–16 (2003).
31. Arifler, D. *et al.* Light scattering from normal and dysplastic cervical cells at different epithelial depths: finite-difference time-domain modeling with a perfectly matched layer boundary condition. *J. Biomed. Opt.* **8**, 484–494 (2003).
32. Arifler, D., MacAulay, C., Follen, M. & Richards-Kortum, R. Spatially resolved reflectance spectroscopy for diagnosis of cervical pre-cancer: Monte Carlo modeling and comparison to clinical measurements. *J. Biomed. Opt.* **11**, 064027 (2006).
33. Drezek, R. *et al.* Autofluorescence microscopy of fresh cervical-tissue sections reveals alterations in tissue biochemistry with dysplasia. *Photochem. Photobiol.* **73**, 636–641 (2001).
34. Lohmann, W., Mussmann, J., Lohmann, C. & Kunzel, W. Native fluorescence of unstained cryo-sections of the cervix uteri compared with histological observations. *Naturwissenschaften* **76**, 125–127 (1989).
35. Mujat, C. *et al.* Endogenous optical biomarkers of normal and human papillomavirus immortalized epithelial cells. *Int. J. Cancer* **122**, 363–371 (2008).
36. DaCosta, R. S., *Wilson*, B. C. & Marcon, N. E. Fluorescence and spectral imaging. *ScientificWorldJournal* **7**, 2046–2071 (2007).
37. Perelman, L. T. Optical diagnostic technology based on light scattering spectroscopy for early cancer detection. *Expert Rev. Med. Devices* **3**, 787–803 (2006).
38. Ramanujam, N. Fluorescence spectroscopy of neoplastic and non-neoplastic tissues. *Neoplasia* **2**, 89–117 (2000).
39. Balas, C. A novel optical imaging method for the early detection, quantitative grading, and mapping of cancerous and precancerous lesions of cervix. *IEEE Trans. Biomed. Eng.* **48**, 96–104 (2001).
40. Pogue, B. W. *et al.* Analysis of acetic acid-induced whitening of high-grade squamous intraepithelial lesions. *J. Biomed. Opt.* **6**, 397–403 (2001).
41. Orfanoudaki, I. M. *et al.* A clinical study of optical biopsy of the uterine cervix using a multispectral imaging system. *Gynecol. Oncol.* **96**, 119–131 (2005).
42. Benavides, J. M. *et al.* Multispectral digital colposcopy for *in vivo* detection of cervical cancer. *Opt. Express* **11**, 1223–1236 (2003).
43. Milbourne, A. *et al.* Results of a pilot study of multispectral digital colposcopy for the *in vivo* detection of cervical intraepithelial neoplasia. *Gynecol. Oncol.* **99**, S67–75 (2005).
44. Huh, W. K. *et al.* Optical detection of high-grade cervical intraepithelial neoplasia *in vivo*: results of a 604-patient study. *Am. J. Obstet. Gynecol.* **190**, 1249–1257 (2004).
45. Alvarez, R. D. & Wright, T. C. Effective cervical neoplasia detection with a novel optical detection system: a randomized trial. *Gynecol. Oncol.* **104**, 281–289 (2007).
46. Ferris, D. G. *et al.* Multimodal hyperspectral imaging for the noninvasive diagnosis of cervical neoplasia. *J. Low. Genit. Tract Dis.* **5**, 65–72 (2001).
47. DeSantis, T. *et al.* Spectroscopic imaging as a triage test for cervical disease: a prospective multicenter clinical trial. *J. Low. Genit. Tract Dis.* **11**, 18–24 (2007).
48. Alvarez, R. D. & Wright, T. C. Jr. Increased detection of high-grade cervical intraepithelial neoplasia utilizing an optical detection system as an adjunct to colposcopy. *Gynecol. Oncol.* **106**, 23–28 (2007).
49. Kendrick, J. E., Huh, W. K. & Alvarez, R. D. LUMA cervical imaging system. *Expert Rev. Med. Devices* **4**, 121–129 (2007).
50. Mirabal, Y. N. *et al.* Reflectance spectroscopy for *in vivo* detection of cervical precancer. *J. Biomed. Opt.* **7**, 587–594 (2002).
51. Chang, S. K. *et al.* Combined reflectance and fluorescence spectroscopy for *in vivo* detection of cervical pre-cancer. *J. Biomed. Opt.* **10**, 024031 (2005).
52. Georgakoudi, I. *et al.* Trimodal spectroscopy for the detection and characterization of cervical precancers *in vivo*. *Am. J. Obstet. Gynecol.* **186**, 374–382 (2002).
53. Mourant, J. R. *et al. In vivo* light scattering measurements for detection of precancerous conditions of the cervix. *Gynecol. Oncol.* **105**, 439–445 (2007).
54. Wang, A., Nammalavar, V. & Drezek, R. Targeting spectral signatures of progressively dysplastic stratified epithelia using angularly variable fiber geometry in reflectance Monte Carlo simulations. *J. Biomed. Opt.* **12**, 044012 (2007).
55. Arifler, D., Schwarz, R. A., Chang, S. K. & Richards-Kortum, R. Reflectance spectroscopy for diagnosis of epithelial precancer: model-based analysis of fiber-optic probe designs to resolve spectral information from epithelium and stroma. *Appl. Opt.* **44**, 4291–4305 (2005).
56. Ramanujam, N. *et al.* Fluorescence spectroscopy: a diagnostic tool for cervical intraepithelial neoplasia (CIN). *Gynecol. Oncol.* **52**, 31–38 (1994).
57. Ramanujam, N. *et al.* Cervical precancer detection using a multivariate statistical algorithm based on laser-induced fluorescence spectra at multiple excitation wavelengths. *Photochem. Photobiol.* **64**, 720–735 (1996).
58. Chang, S. K. *et al.* Optimal excitation wavelengths for discrimination of cervical neoplasia. *IEEE Trans. Biomed. Eng.* **49**, 1102–1111 (2002).
59. Weingandt, H. *et al.* Autofluorescence spectroscopy for the diagnosis of cervical intraepithelial neoplasia. *Br. J. Obstet. Gynecol.* **109**, 947–951 (2002).
60. Drezek, R. *et al.* Understanding the contributions of NADH and collagen to cervical tissue fluorescence spectra: modeling, measurements, and implications. *J. Biomed. Opt.* **6**, 385–396 (2001).
61. Chang, S. K., Arifler, D., Drezek, R., Follen, M. & Richards-Kortum, R. Analytical model to describe fluorescence spectra of normal and preneoplastic epithelial tissue: comparison with Monte Carlo simulations and clinical measurements. *J. Biomed. Opt.* **9**, 511–522 (2004).
62. Georgakoudi, I. *et al.* NAD(P)H and collagen as *in vivo* quantitative fluorescent biomarkers of epithelial precancerous changes. *Cancer Res.* **62**, 682–687 (2002).
63. Brookner, C. K., Utzinger, U., Staerkel, G., Richards-Kortum, R. & Mitchell, M. F. Cervical fluorescence of normal women. *Lasers Surg. Med.* **24**, 29–37 (1999).
64. Nordstrom, R. J., Burke, L., Niloff, J. M. & Myrtle, J. F. Identification of cervical intraepithelial neoplasia (CIN) using UV-excited fluorescence and diffuse-reflectance tissue spectroscopy. *Lasers Surg. Med.* **29**, 118–127 (2001).
65. Pavlova, I., Williams, M., El-Naggar, A., Richards-Kortum, R. & Gillenwater., A. Understanding the biological basis of autofluorescence imaging for oral cancer detection: High resolution fluorescence microscopy in viable tissue. *Clin. Cancer Res.* **8**, 2396–2404 (2008).
66. Collier, T., Lacy, A., Richards-Kortum, R., Malpica, A. & Follen, M. Near real-time confocal microscopy of amelanotic tissue: detection of dysplasia in *ex vivo* cervical tissue. *Acad. Radiol.* **9**, 504–512 (2002).
67. Evans, J. A. & Nishioka, N. S. Endoscopic confocal microscopy. *Curr. Opin. Gastroenterol.* **21**, 578–584 (2005).
68. MacAulay, C., Lane, P. & Richards-Kortum, R. *In vivo* pathology: microendoscopy as a new endoscopic imaging modality. *Gastrointest. Endosc. Clin. N. Am.* **14**, 595–620, xi (2004).
69. Collier, T., Shen, P., de Pradier, B. & Richards-Kortum, R. Near real time confocal microscopy of amelanotic tissue: dynamics of aceto-whitening enable nuclear segmentation. *Opt. Express* **6**, 40–48 (2000).
70. Luck, B. L., Carlson, K. D., Bovik, A. C. & Richards-Kortum, R. R. An image model and segmentation algorithm for reflectance confocal images of *in vivo* cervical tissue. *IEEE Trans. Image Process.* **14**, 1265–1276 (2005).
71. Sung, K. B. *et al.* Near real time *in vivo* fibre optic confocal microscopy: sub-cellular structure resolved. *J. Microsc.* **207** (Pt 2), 137–145 (2002).
72. Tan, J., Delaney, P. & McLaren, W. J. Confocal endomicroscopy: a novel imaging technique for *in vivo* histology of cervical intraepithelial neoplasia. *Expert Rev. Med. Devices* **4**, 863–871 (2007).
73. Kiesslich, R. *et al.* Confocal laser endoscopy for diagnosing intraepithelial neoplasias and colorectal cancer *in vivo*. *Gastroenterology* **127**, 706–713 (2004).
74. Sokolov, K. *et al.* Optical systems for *in vivo* molecular imaging of cancer. *Technol. Cancer Res. Treat* **2**, 491–504 (2003).
75. Hsiung, P. L. *et al.* Detection of colonic dysplasia *in vivo* using a targeted heptapeptide and confocal microendoscopy. *Nature Med.* **14**, 454–458 (2008).
76. Koyama, Y. *et al. In vivo* molecular imaging to diagnose and subtype tumors through receptor-targeted optically labeled monoclonal antibodies. *Neoplasia* **9**, 1021–1029 (2007).
77. Adams, K. E. *et al.* Comparison of visible and near-infrared wavelength-excitable fluorescent dyes for molecular imaging of cancer. *J. Biomed. Opt.* **12**, 024017 (2007).
78. Aaron, J. *et al.* Plasmon resonance coupling of metal nanoparticles for molecular imaging of carcinogenesis *in vivo*. *J. Biomed. Opt.* **12** (2007).
79. Sokolov, K. *et al.* Real-time vital optical imaging of precancer using anti-epidermal growth factor receptor antibodies conjugated to gold nanoparticles. *Cancer Res.* **63**, 1999–2004 (2003).
80. Wu, X. *et al.* Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. *Nature Biotechnol.* **21**, 41–46 (2003).
81. Nida, D. L., Rahman, M. S., Carlson, K. D., Richards-Kortum, R. & Follen, M. Fluorescent nanocrystals for use in early cervical cancer detection. *Gynecol. Oncol.* **99**, S89–S94 (2005).
82. Cuenca, A. G. *et al.* Emerging implications of nanotechnology on cancer diagnostics and therapeutics. *Cancer* **107**, 459–466 (2006).
83. Chang, E., Thekkek, N., Yu, W. W., Colvin, V. L. & Drezek, R. Evaluation of quantum dot cytotoxicity based on intracellular uptake. *Small* **2**, 1412–1417 (2006).
84. Nitin, N., Javier, D. J., Roblyer, D. M. & Richards-Kortum, R. Widefield and high-resolution reflectance imaging of gold and silver nanospheres. *J. Biomed. Opt.* **12**, 051505 (2007).
85. Aslan, K., Zhang, J., Lakowicz, J. R. & Geddes, C. D. Saccharide sensing using gold and silver nanoparticles — a review. *J. Fluoresc.* **14**, 391–400 (2004).
86. Vizcaino, A. P. *et al.* International trends in incidence of cervical cancer: II. Squamous-cell carcinoma. *Int. J. Cancer* **86**, 429–435 (2000).
87. Roblyer, D., Richards-Kortum, R., Park, S. Y., Adewole, I. & Follen, M. Objective screening for cervical cancer in developing nations: lessons from Nigeria. *Gynecol. Oncol.* **107**, S94–97 (2007).
88. Muldoon, T. *et al.* High resolution imaging in Barrett's esophagus: a novel, low-cost endoscopic microscope. *Gastrointest. Endosc.* (in the press).
89. Visual inspection with acetic acid for cervical-cancer screening: test qualities in a primary-care setting. University of Zimbabwe/JHPIEGO Cervical Cancer Project. *Lancet* **353**, 869–873 (1999).
90. Denny, L., Kuhn, L., Pollack, A., Wainwright, H. & Wright, T. C. Jr. Evaluation of alternative methods of cervical cancer screening for resource-poor settings. *Cancer* **89**, 826–833 (2000).
91. Belinson, J. L. *et al.* Cervical cancer screening by simple visual inspection after acetic acid. *Obstet. Gynecol.* **98**, 441–444 (2001).
92. Cronje, H. S. *et al.* A comparison of four screening methods for cervical neoplasia in a developing country. *Am. J. Obstet. Gynecol.* **188**, 395–400 (2003).
93. Sankaranarayanan, R. *et al.* Accuracy of visual screening for cervical neoplasia: Results from an IARC multicentre study in India and Africa. *Int. J. Cancer* **110**, 907–913 (2004).

Acknowledgements

The authors would like to acknowledge the support of a National Institutes of Health Bioengineering Research Partnerships grant (CA103830).

Competing interests statement

The authors declare competing financial interests: see web version for details.

DATABASES

Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
EGFR
National Cancer Institute:
http://www.cancer.gov/
cervical cancer

FURTHER INFORMATION

R. Richard-Kortum's homepage:
http://cohesion.rice.edu/engineering/kortum
Beyond Traditional Borders:
http://beyondtraditionalborders.rice.edu

ALL LINKS ARE ACTIVE IN THE ONLINE PDF