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Aspects of photodetection in cervical and ovarian neoplasia

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Chapter 9

Tumor staging with Double Ratio fluorescence imaging; a Monte Carlo study

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Abstract

Fluorescence imaging of cervical dysplasia may be an accurate and easy alternative to conventional colposcopy. In addition to colposcopy, it may quantify the thickness of the neoplastic layer, indicating the grade of cervical cancer, as was shown in chapter 8. The aim of this study was to gain more insight in the dependency of the Double Ratio on layered geometries, as is the clinical situation in practice. A Monte-Carlo calculation method was used which allowed the composition of different geometries with embedded fluorescent volumes representing the neoplastic tissue. Several configurations were defined; all consisting of a fluorescent cube embedded in a fluorescent layer. The optical properties for the excitation light and for several layer thicknesses were varied in these simulations. The values and geometrical assumption used for the Monte Carlo simulation were used to investigate a concept that may give insight in the correlation between DR and stage of the disease.

From the simulations and the clinical study of chapter, 8 it seems possible to use the DR technique to localize and grade cervical intraepithelial neoplasia.

Introduction

It is important to recognize and grade cervical cancer accurately and at an early stage in order to be able to determine a suitable treatment strategy. Currently used diagnostic methods are usually non-specific, dependent on the skills of the gynecologist or are too expensive to be used routinely. An alternative or complementary technique may be fluorescence imaging. Fluorescence imaging is a non-invasive technique that is increasingly used for the localization and staging of (early stage) tumors and tumor metastases. The technique is based on the fluorescent properties of either an intrinsic tissue specific dye or a selective exogenously administered dye. The tissue is illuminated with light at a specific wavelength, for most dyes this will be in the blue part of the spectrum. Broad band fluorescent light that escapes from the tissue can be imaged using filtered red-sensitive cameras. The image that is captured by the camera contains information about the composition of the whole target volume. Penetration of the blue light that is usually used for excitation of the dye is limited to superficial layers. The technique is therefore particularly suitable for imaging of superficial cervical intraepithelial neoplastic layers.

Besides the distribution and concentration of the fluorescent dye, the fluorescence data also depend on tissue optical properties and excitation and detection geometry. Several techniques have been developed to eliminate the tissue and device artifacts¹⁻⁵. The simplest way of fluorescence diagnostics is by just looking at the emitted fluorescence. By taking the ratio of the fluorescence signal of the photosensitizer and the fluorescence of the intrinsic tissue components (background) it is possible to eliminate influences of excitation and detection geometry. This ratio, normalized on a ratio taken without fluorophores presents is called the normalized fluorescence ratio³. The ratio of the two ratios measured with two excitation wavelengths is called the Double Ratio (DR)⁴. This double ratio is in theory independent of the optical properties of the tissue and independent of the excitation and detection geometry of the device. For the derivation of the double ratio, several assumptions were made. A semi-infinite medium is assumed to contain a homogenous distribution of photosensitizer. Both these assumption may be violated when measuring on the cervix.

Epithelial neoplasia grows from the boundary between the epithelial and stromal layer towards the surface and will, with progressing stage, fill the monitored volume gradually. The thickness of the layer will thus have an effect on the measured average fluorescence in the tissue. This was confirmed by the results of the clinical study as described in chapter 8. A clear correlation was found between the DR value and the CIN grade.

A better understanding of the interrelations between DR, thickness of the neoplastic layer and penetration depth of the excitation light is however necessary to further develop DR fluorescence measurements into a dedicated tool for staging of CIN. In this chapter, these relations were investigated with a Monte Carlo model. Furthermore, we will try to correlate the results of this chapter with the clinical results of chapter 8.

Methods

For the calculations, we adjusted a Monte Carlo program, based on the code of Keijzer *et al.*⁶ adapted by Lucassen *et al.*⁷. The program uses a variance reduction method that is called survival weighting. The photons are transported through the medium with a certain weight. At each interaction, a fraction $\mu_a/(\mu_a+\mu_s)$ of the weight is deposited while the remaining fraction continues. The program was adjusted to enable fluorescence imaging simulation. First, the distribution of the excitation light is calculated using the source type and dimensions and the optical properties at the excitation wavelength of the medium as input parameters. In the remaining of this chapter this will be called the 'excitation Monte Carlo'. In this excitation Monte Carlo, the contribution of the absorption of the fluorophores to the total absorption is not taken into account. Light that is absorbed in the medium is scored in a (z, r) matrix that is used to assign weight to the fluorescence sources. A separate input file that contains the distribution of the fluorophores is used as source file for calculation of the fluorescent light distribution, the

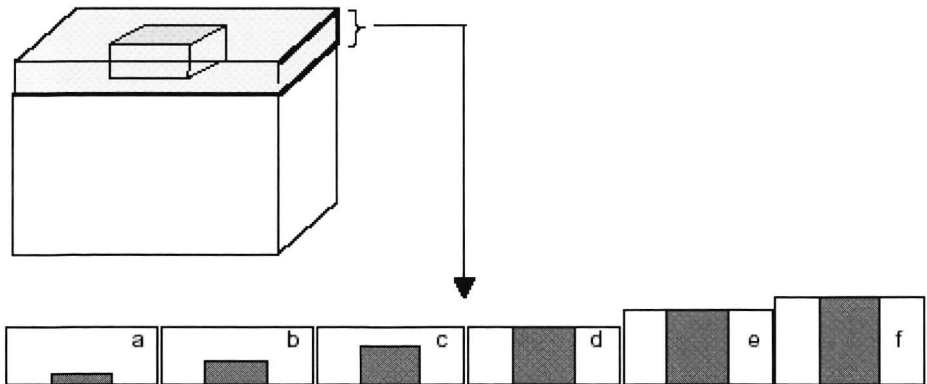


Figure 1: Different configurations of the two-layered geometry with the neoplastic volume embedded (gray square).

'fluorescence Monte Carlo'. The fraction of photons that escapes the medium from the top layer within a certain escape angle is scored in a matrix. Relative fluorescence levels

are determined for semi-infinite double layer geometries (representing the epithelial layer and the stromal layer) with embedded square geometries representing neoplastic areas. The x/y plane of the cube has dimensions of 2.5cm x 2.5cm, the x/y plane of the 'tumor' has sides of 1.25cm x 1.25 cm.

For the simulations, the thickness of the neoplastic area that originates at the boundary of the two layers was varied. The following progression was assumed in the simulations: the epithelial layer is first set to 0.5 mm⁸. The 'neoplasia' progresses from the boundary between the two layers into the top layer (figures 1a-1d) When its thickness equals 0.5 mm (figure 1d), we let the total epithelial layer increases in thickness from 0.5 to 1.3 mm (figures 1d to 1f) according to the findings of Abdul-Karim *et al.*⁹

Input parameters:

Calculation of the excitation light.

To our knowledge, the optical properties of cervical tissue in the blue (excitation) light range are not known. Three different sets of optical parameters were chosen to obtain different optical penetration depths (δ), which we calculated with equation 1:

$$\delta = \sqrt{\frac{1}{3\mu_a(\mu_a + \mu_s(1-g))}} \quad (1)$$

Where μ_a = absorption coefficient (cm⁻¹),

μ_s = scattering coefficient (cm⁻¹),

g = anisotropy factor (-)

The different combinations of optical parameters with their corresponding theoretical penetration depths δ are given in table 1:

Table 1: input (optical) parameters for the excitation Monte Carlo's

	μ_a (cm ⁻¹)	μ_s (cm ⁻¹)	g (-)	δ (μ m)
excitation source 1	100	400	0.87	47
excitation source 2	15	400	0.87	180
excitation source 3	2	200	0.87	771

The optical properties that are used for excitation source 2 are in the range of the absorption coefficient and a scattering coefficient for blue light as found by Keijzer *et al.*¹⁰ of aorta tissue.

An incident light beam with a flat intensity profile, a diameter of 5 cm and a total energy of 100 J was defined.

Calculation of the fluorescence light.

The optical properties for the fluorescence Monte Carlo's were chosen to be in the range as found by Hornung et al.⁴:

Layer 1: μ_a : 0.15 cm⁻¹, μ_s 90 cm⁻¹, g: 0.9, n: 1.37, thickness: 500-1300 μ m

Layer 2: μ_a : 1 cm⁻¹, μ_s 150 cm⁻¹, g: 0.9, n: 1.37, thickness: >5 cm.

For the fluorescent 'neoplastic volume', the optical properties of layer 1 were used.

During the clinical study of chapter 8, we obtained the 'red green fluorescence ratio' (FR) as described by Profio *et al.*² on different locations on the cervix. In short, the FR is defined as the ratio between the fluorophore fluorescence and the autofluorescence from one excitation wavelength. We assumed this ratio to be proportional to the amount of fluorophores. Because the autofluorescence intensity and the optical properties of the tissue for the excitation and emission wavelengths influence the ratio it is only indicative for relative concentration differences on one tissue type in a single patient. The value that was measured on tissue that was colposcopically assessed abnormal was compared to values measured on tissue judged as normal. The selectivity obtained in this way was approximately 8. This selectivity was used in the calculation of the double ratio from the Monte Carlo results.

The results of the calculations will be compared with the clinical results (chapter 8). In the clinical study, a DR of 2 was associated with CIN I, a DR of 3 was associated with CIN II, a DR between 3 and 4.5 was associated with CIN III.

Results

Figure 1 shows the intensity profiles of the three different excitation profiles that were used. 'Excitation source 1' has a very limited penetration, less than the 500 μ m that we chose for the epithelial layer, 'excitation source 2' will penetrate beyond the 500 μ m layer into the second layer while 'excitation source 3' penetrates the 'tissue' up to a depth beyond 1000 μ m.

For each of the figures 2, 3 and 4, only one 'excitation source' is used per data set. In this way, the penetration depth of the two excitation wavelengths is equal, as is assumed in the derivation of the DR theory⁴. The selectivity of the 'photosensitizer' and the 'exci-

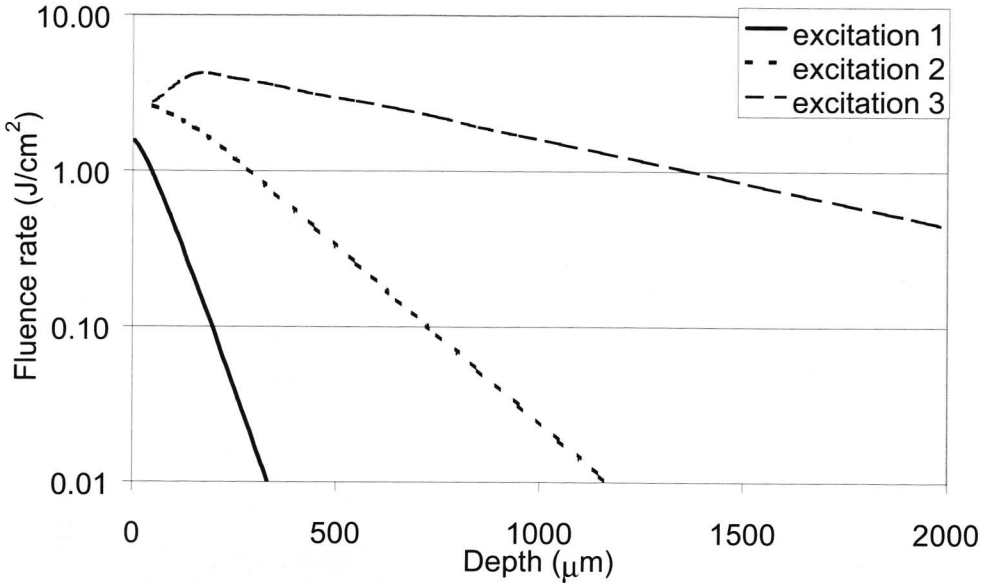


Figure 1: excitation profiles for the different excitation sources'.

tation efficiency' is equal for these data sets.

Figure 2 shows the DR as function of thickness of the neoplastic volume after excitation with excitation source 1.

The first 200 μm hardly contributes to the DR signal because of the limited penetration depth of the excitation light. When the tumor grows into the monitored area, the double ratio rises quickly until 500 μm is reached. From that point, both the total layer and the tumor increase in thickness, which, as seen from the top surface, is equal to the addition of fluorescence below the layer of 500 μm . Although the DR seems saturated, the plateau phase is caused by the limited penetration of the excitation light.

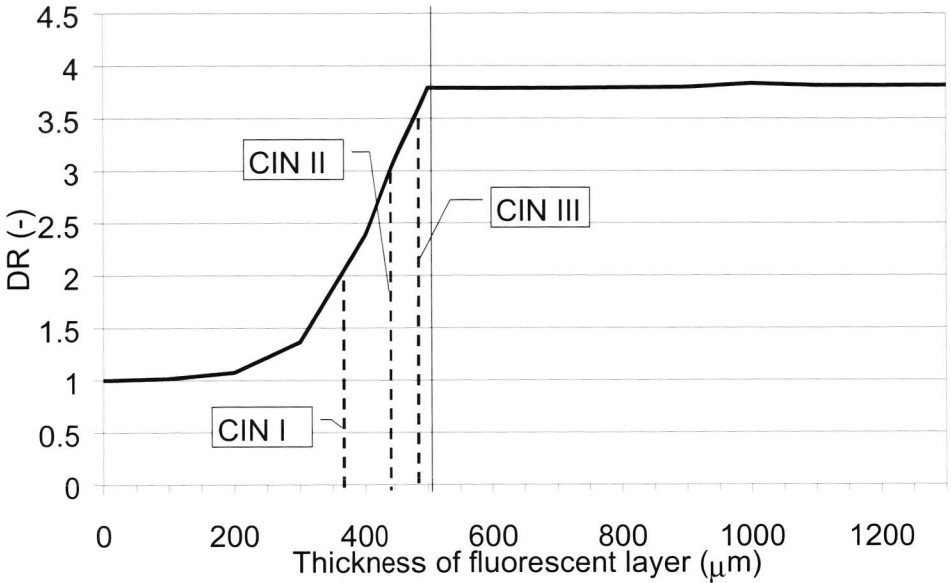


Figure 2: DR as function of thickness of the embedded 'tumor'. Excitation source 1 was used in the simulations. On the left side of the vertical line, the 'tumor' is growing in the 500 μm top layer. On the right side, the 'tumor' and the top layer are both increasing in thickness. The CIN grades corresponding to the DR values (see chapter 8) are added.

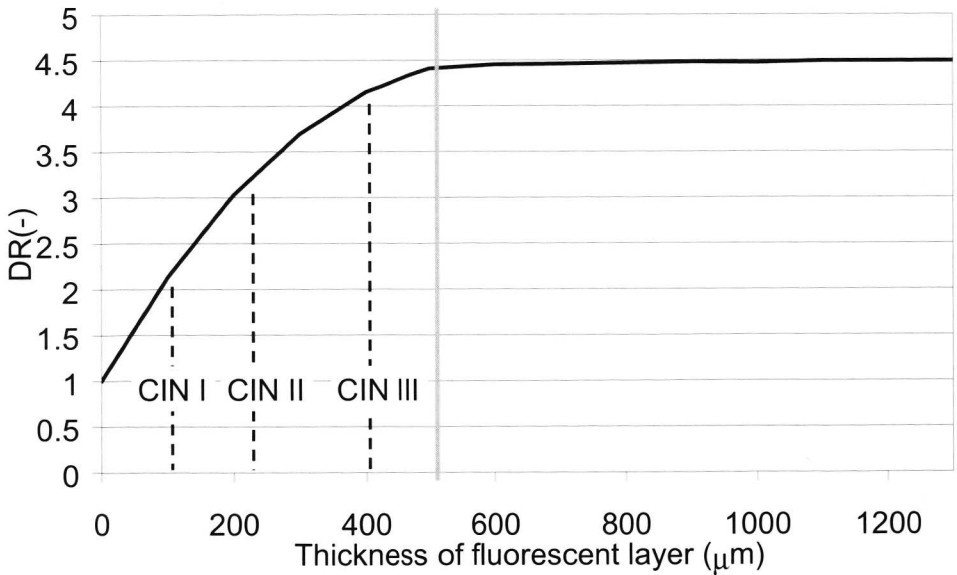


Figure 3: Interrelationships of Double ratio value, CIN grade (chapter 8) and layer thickness as predicted with the Monte Carlo simulations. Excitation source 2 is used for excitation.

Figure 3 shows the DR as function of tumor grade after excitation with excitation source 2. Again, by combining figure 3 of chapter 8, which shows the relation of the DR with CIN grade, with figure 3 on page 118 which was obtained with excitation source 2, we can estimate the thickness of the layers at different CIN grades

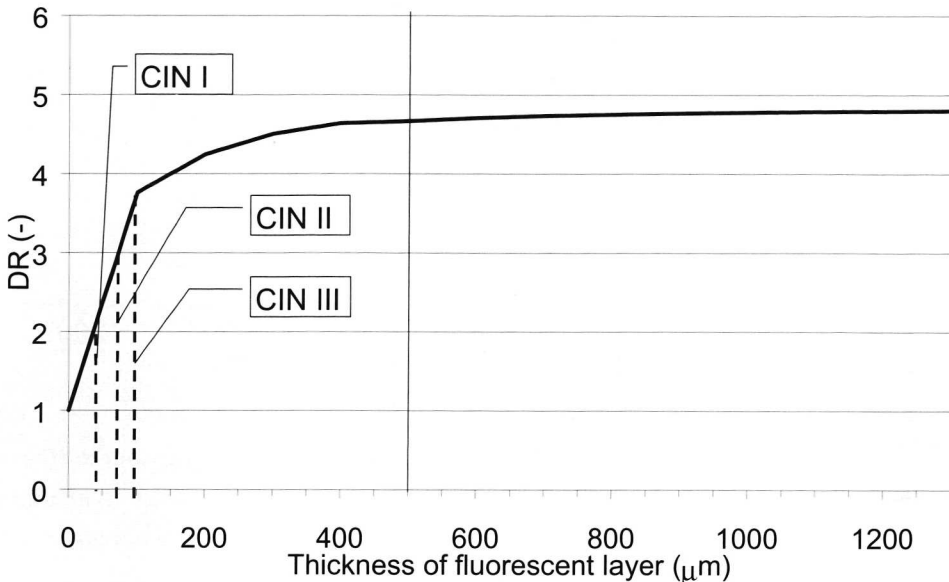


Figure 4: DR as function of thickness of the embedded 'tumor'. Excitation source 3 was used in the simulations. On the left side of the vertical line, the 'tumor' is growing in the 500 μm layer. On the right side, the 'tumor' and the top layer are both increasing in thickness. Again, the CIN grades corresponding to the DR values (see chapter 8) are added

The influence of the first layers on the DR signal is relatively large because of a deep penetration of the excitation light and the non-linear relation of the DR with the 'photosensitizer' concentration.

For figure 5, the DR was calculated using two excitation sources (1 and 3) to investigate whether the sensitivity of the technique could be improved by using one excitation source that penetrates the deeper layers and one excitation source with which only the superficial layers are monitored. The DR is obtained by dividing the SR of excitation source 3 by the SR of excitation source 1.

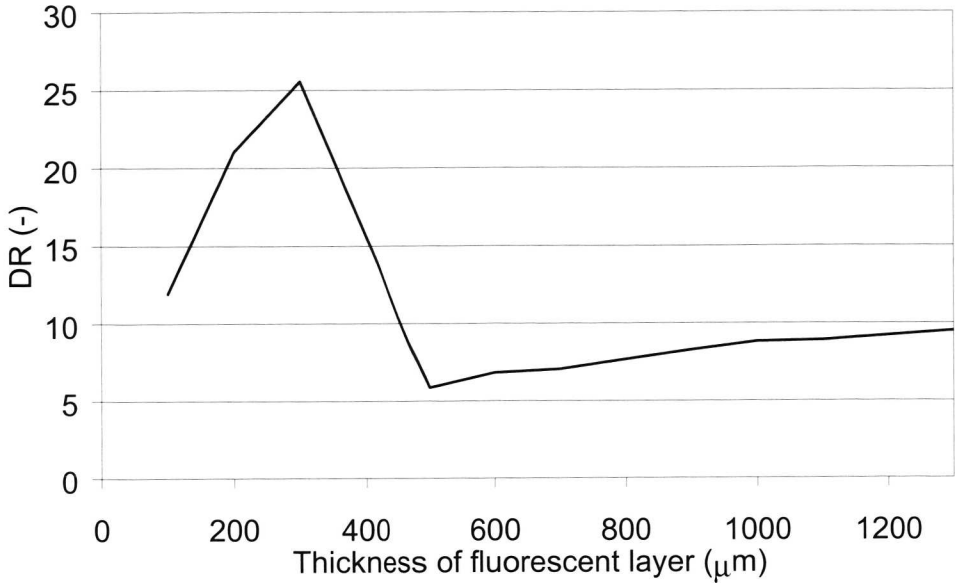


Figure 5: DR calculated with a ratio of the SR's obtained with excitation source 3 and 1.

The first 300 μm is only monitored by the deeper penetrating excitation source (the numerator) hence the rapid rise of the DR. When the layer is thick enough to also influence the denominator, the DR value drops until a thickness of 500 μm whereafter only the numerator increases.

Discussion

Fluorescence Monte Carlo calculations were performed to evaluate the possibility to use Double ratio measurements for grading of cervical intraepithelial neoplasms and, with that, to understand the results of chapter 8. For this we defined a two-layered structure, representing the fluorescent epithelial layer and an underlying stromal layer, and an embedded fluorescent cube, representing the neoplastic area. By performing simulations with various layer thicknesses we demonstrated the dependency of the DR on layer thickness for various different excitation wavelengths.

In the mathematical model we have made several assumptions for the optical properties and thicknesses of the layers, the development of CIN etc. Also the representation of the neoplastic volume by a perfectly sharp delineated cube is far from the realistic clinical situation. Moreover, the optical properties of cervical tissue are not known and the values used in the Monte Carlo simulation were a best guess. However, the values and geometrical assumption used for the Monte Carlo simulation were used to investigate a con-

cept that may give insight in the the correlation between DR and stage of the disease. The validity of this concept does not depend on the exact values of the input parameters as long as the relative relations are reasonable.

The use of two excitation wavelengths with different penetration depths turned out to be of no clinical value with the geometry and parameters that were used for these simulations. In addition, the resulting ratio does no longer comply with the assumptions that were made during the derivation by Sinaasappel *et al.* and will be sensitive to all the factors the DR is supposed to correct for. Determination of layer thickness by using the DR technique can only be performed by using two excitation wavelengths at which the tissue optical properties are approximately equal.

From the clinical study of chapter 8 and the mathematical evaluation we may conclude that by using the double ratio fluorescence measurement technique, it may be possible to localize and differentiate non-invasively between different groups of CIN.

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