Identification of human breast tissues by near infrared spectroscopy

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Introduction

Breast cancer presents varied histological types with different survival rates. As the therapeutic approach is diversifying, most modern studies take into account the histological type and histoprognostical grade (SBR system), as prognostic indicators for the therapeutic choice.

As reported in a previous paper, near infrared (NIR) spectroscopy can discriminate between normal and carcinomatous human breast tissues.[1] The aim of the present study is to assess the value of NIR spectroscopy as a prognostic tool in breast cancer evaluation by studying the correlation between pathological and spectral data.

Material

Four different types of breast tissue samples were obtained from surgical specimens:

1. Reference samples: normal breast tissue from seven non-cancerous patients.
2. Control samples: normal breast tissue from 35 cancerous patients, taken at a distance from the tumour.
3. Carcinomatous tissue from invasive carcinomas of special types:
   * Invasive Lobular Carcinoma 10 patients
   * Colloid Carcinoma 6 patients.
4. Carcinomatous tissue from Invasive Ductal Carcinoma
   Grades: * SBR I 5 patients
   * SBR II 25 patients
   * SBR III 10 patients.

Method

Near infrared spectroscopy

From each tissue sample ($1 \times 1 \times 0.2$ cm thick) 3 to 14 (15 micrometres thick) frozen sections were mounted on a coverslip and air-dried. The coverslip was centered between a glass fibre filter (Millipore AP40047) and a 55 mm diameter glass window and placed in a standard reflectance

sample cup. NIR spectra of sections were recorded every 2 nm from 1100 to 2500 nm using a spectrometer PS Co 6250 (NIRSystems, Silver Spring, MD) working in a single beam mode.

Histopathology

As histological controls we used one frozen and one paraffin section, stained by routine hematoxylin-eosin technique. The diagnosis was formulated according to the WHO classification. The SBR grading system was estimated from the variations in glandular differentiation, size and shape of the nuclei and mitotic counts.

The WHO nomenclature and the SBR grading system [grades I, II (IIA, IIB and IIC) and III) allow prognostication about the aggressivity of the tumour: SBR I invasive ductal carcinoma has a good prognosis with a mean 5 year survival of about 80%, while the 5 year survival of SBR III is about 25%. Colloid carcinoma has a prognosis similar to that of SBR I and invasive lobular carcinoma a little worse than SBR I.[2]

Results and discussion

The 2nd derivative reflectance spectra was examined in order to evaluate the presence of the 10 peaks in the four zones. The NIR spectroscopy results were expressed in mean frequencies (%) in the four selected zones (i.e. frequency with which the expected peaks were observed in the selected zones).

Table 1 and Figure 1 show the position of the selected zones and peaks on NIR spectroscopy 2nd derivative spectra of normal reference human breast tissues.

Table 2 summarises the results obtained in 56 cases of breast cancers. In comparison with the control tissue, all the cancers show a very low mean frequency in the C zone. In the A, B and D zones variable degrees of abnormalities are found, more pronounced in SBR II-B and SBR-III invasive ductal carcinoma.

Figure 2 illustrates the 2nd derivative spectra of two invasive ductal carcinomas, respectively, of SBR grades I and III : the mean frequencies in the A, B and D zones of the SBR I are closer to the data of normal control tissue and definitely different from SBR III carcinoma. However, the mean frequencies in the C zone are similar for both grades.

Relating to invasive ductal carcinoma, the results seem to be correlated to the histological grading system:

- Carcinomas with good prognosis (SBR I) show results closer to normal while carcinomas with bad prognosis (SBR III) show lower frequencies.
- In the intermediate group (SBR II) lower frequencies are seen in the higher grades (SBR II-B and SBR II-C).

Table 3 summarises the results of the mean frequencies of histologically normal tissue:
(a) reference normal tissue from non-cancer patients
(b) control normal tissue from cancer patients.

Table 1. Position (nm) of selected zones and peaks on NIR spectroscopy 2nd derivative spectra of normal reference human breast tissues.

Zones	A		B			C		D		
Peaks	1	2	3	4	5	6	7	8	9	10
from	1208	1240	1746	1764	1786	2012	2044	2326	2346	2368
to	1210	1242	1750	1768	1788	2016	2048	2326	2348	2368

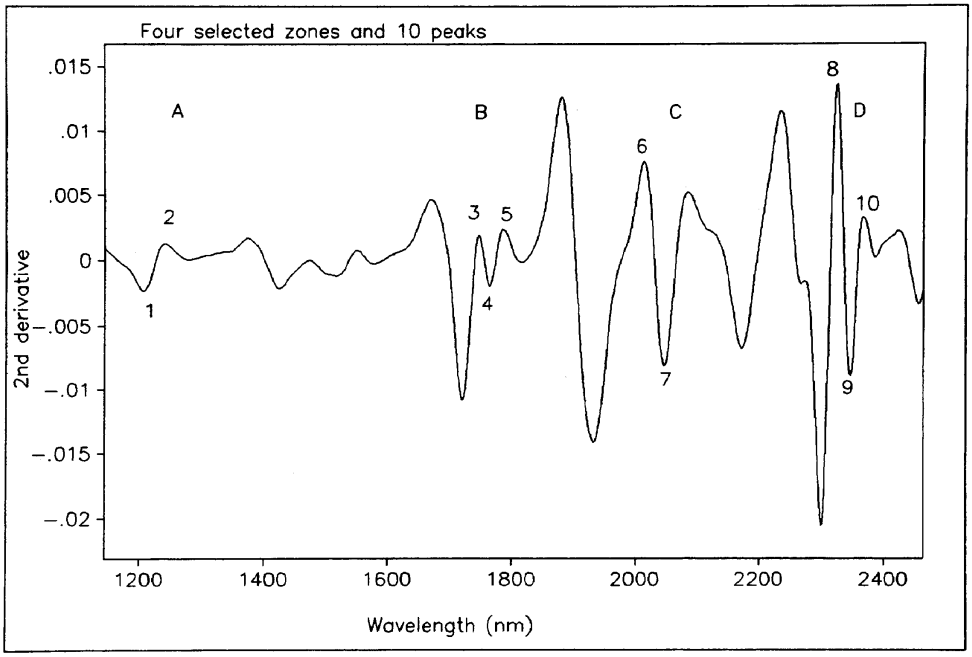

Figure 1. Position (nm) of selected zones and peaks on NIR spectroscopy 2nd derivative spectra of normal reference human breast tissues.

Table 2. Mean frequencies (%) obtained in 56 cases of breast cancers.

Breast tissues (N cases, N sections) Zones	A	B	C	D
Control normal tissue [taken at a distance from the tumour (35, 313)]	85	89	85	85
Special types of breast cancer:				
—Invasive Lobular Carcinoma (10, 74)	31	46	8	43
—Colloid Carcinoma (6, 48)	22	33	6	29
Invasive Ductal Carcinoma:				
SBR I (5, 25)	92	96	0	92
SBR II A (9, 54)	81	91	4	87
SBR II B (8, 67)	28	61	9	1
SBR II C (8, 67)	2	8	10	12
SBR III (10, 94)	4	20	8	3

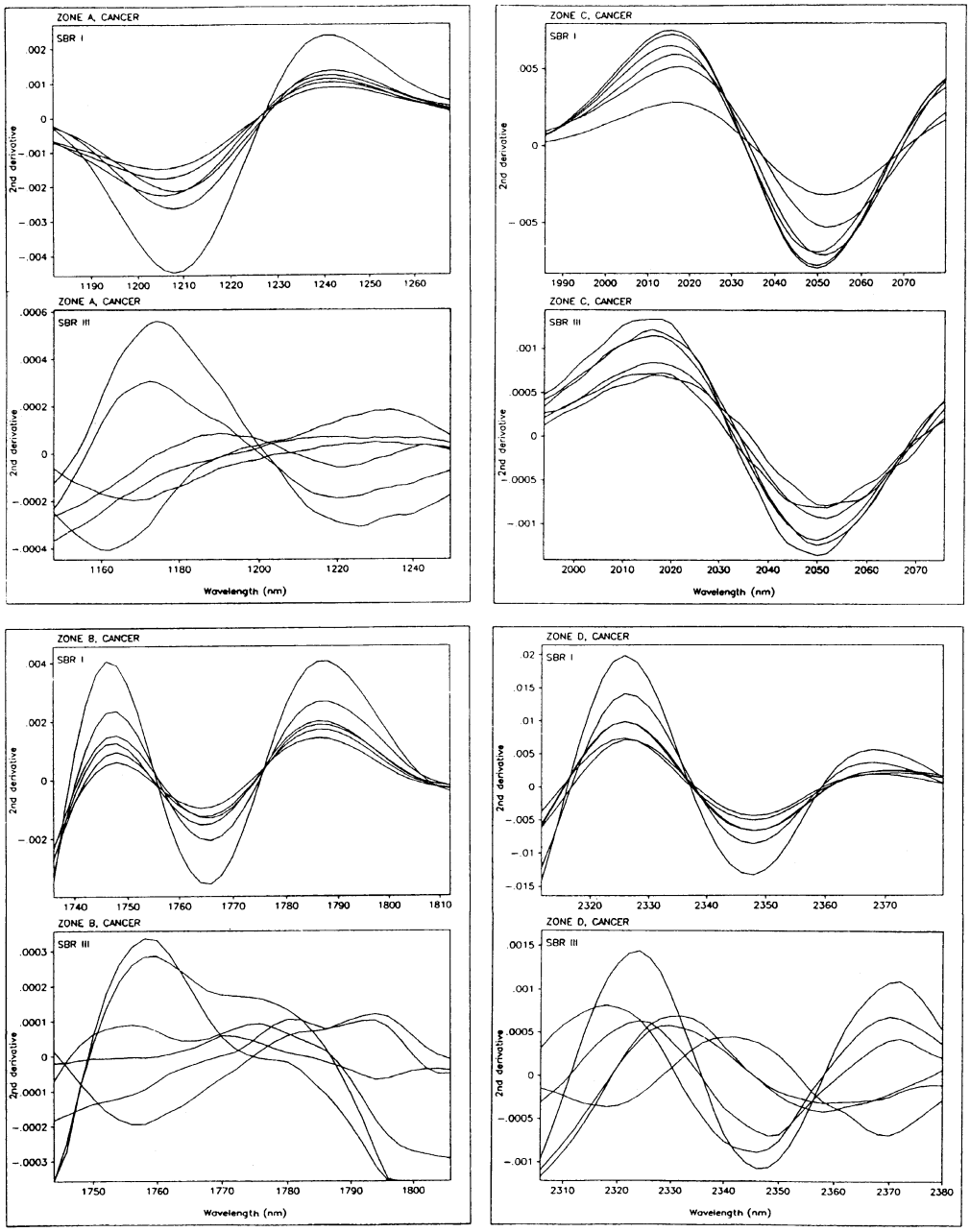

Figure 2. 2nd derivative spectra of SBR I and SBR II invasive ductal carcinomas.

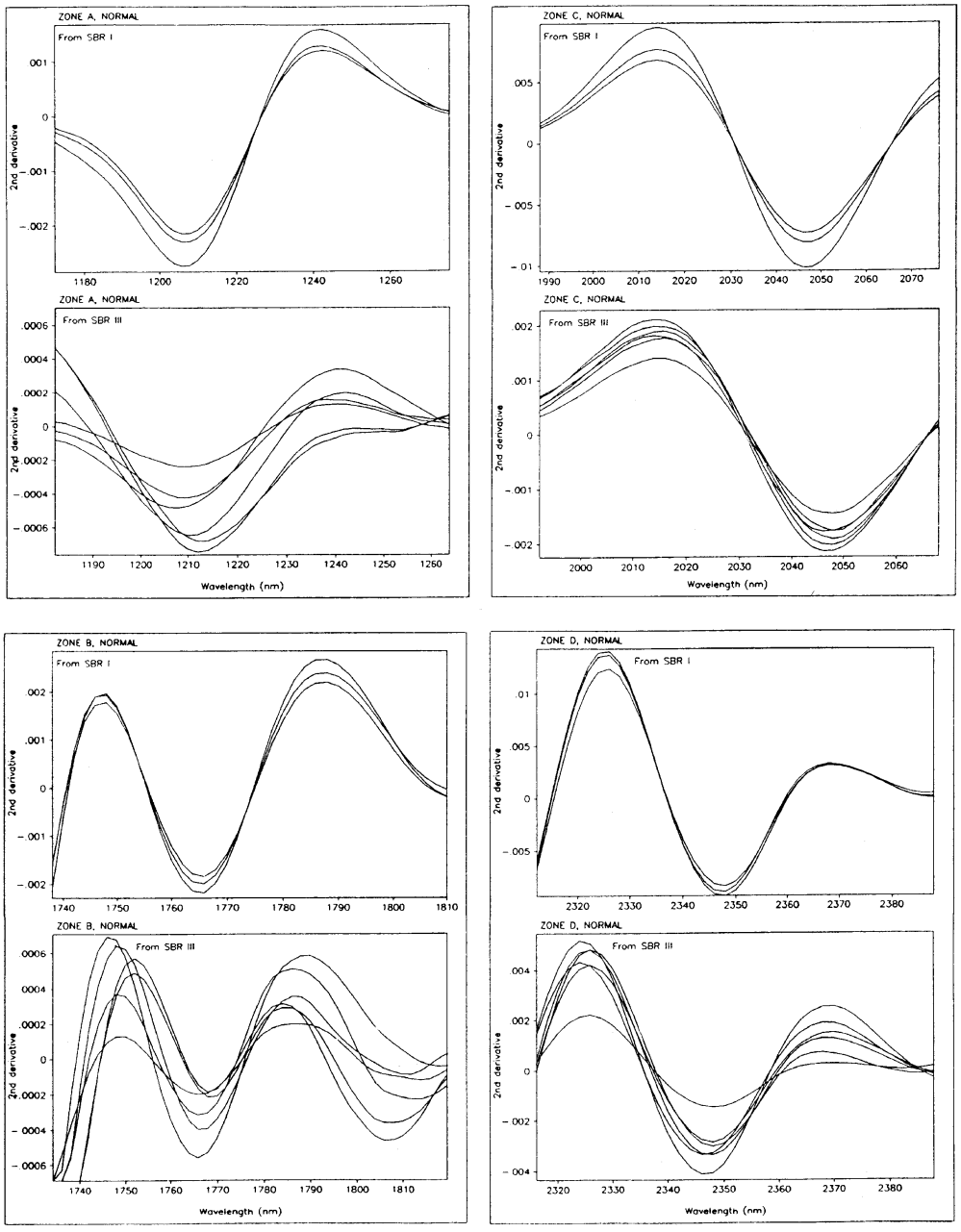

Figure 3. 2nd derivative spectra of control normal breast tissue taken at a distance from the tumour.

Table 3. Mean frequencies (in %) of histologically normal breast tissues.

Breast tissues (N cases, N sections) Zones	A	B	C	D
Reference normal tissue from non-cancer patients (7, 60)	92	100	100	100
Control tissue from cancer patients				
—Invasive Lobular Carcinoma (5, 24)	100	100	100	100
—Colloid Carcinoma (3, 30)	93	97	97	97
Invasive Ductal Carcinoma:				
SBR I (4, 22)	91	96	100	91
SBR II A (6, 59)	93	97	88	98
SBR II B (6, 64)	78	94	84	70
SBR II C (4, 46)	74	72	78	67
SBR III (7, 68)	71	78	66	78

In all series the mean frequency is high but is found to be lower in the case of SBR II-C and SBR III patients. The 2nd derivative spectra are less regular and show a higher deviance from normal (reference samples from non-cancer patients).

Conclusions

- NIR spectroscopy can distinguish carcinomatous from normal human breast tissues.
- NIR spectroscopy can classify invasive ductal carcinomas in different subtypes, corresponding to the histopathological SBR grading system.
- In SBR II and III (high grade ductal carcinoma) NIR spectroscopy of control tissue taken at a distance from the cancer seem to give results definitely different from those of reference normal tissue.

References

1. J. Wallon, S.H. Yan, J. Tong, M. Meurens and J. Haot, *Appl. Spectrosc.* **48(2),** 190 (1994).
2. C.W. Elston, "Grading of Invasive Carcinoma of the Breast", *in Diagnostic Histopathology of the Breast*, Ed by D.L. Page and T.J. Anderson. Churchill Livingstone, Edinburgh, Ch. 17, pp. 300–311 (1987).