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An original stereomicroscopic analysis of the mammary glandular tree

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Summary. In the 1970s, Wellings developed and reported extensively on a technique for a three-dimensional (3D) analysis of breast lesions. Drawbacks of this subgross sampling technique were that it was laborious, rather time-consuming and only allowed prospective studies. Furthermore, the stereomicroscopic aspect of the lesions studied was not diagnostic and each sample had to be studied histologically after paraffin embedding to determine the diagnosis. The present study introduces an original method enabling the exploration of the 3D structure of the mammary glandular tree from a paraffin-embedded sample. This procedure is quicker than the Wellings' technique, permits retrospective study and enables a 3D analysis of previously identified histological structures. Stereomicroscopic aspects of non-malignant lesions such as single multiple or metaplastic cysts, adenosis, ductal-lobular hyperplasia and malignant in situ neoplasms are illustrated. Our results confirm Wellings' concept that most minimal lesions arise in the terminal ductulo-lobular units. We also show that ductal carcinoma in situ may grow continuously by extending through the glandular tree but may also have a multifocal or stepwise progression in some cases.

Key words: Breast – Histological techniques – Three-dimensional vision – Carcinoma in situ

Introduction

A fundamental part of our knowledge of breast pathology is based on studies using three-dimensional (3D) stereomicroscopic analysis techniques. These techniques have enhanced the understanding of the morphology and histogenesis of human breast diseases. Stereomicroscopic investigations, have shown that many minimal breast lesions arise in the terminal ductulo-lobular unit (TDLU) rather than in larger ducts (Tanaka and Oota 1970; Wellings and Jensen 1973). The concept of the multifocality of breast cancer, which is strongly influencing recent therapeutic strategies, has been demonstrated by studies correlating subgross, radiographic, stereomicroscopic and microscopic findings (Egan 1982; Holland et al. 1985; Hutter and Kim 1971; Lagios 1977; Wellings et al. 1975). These authors used various methods: graphic 3D reconstruction from serial histological sections (Ohuchi et al. 1984, 1985), direct visualization of the mammary glandular tree in thick tissue sections (Alpers and Wellings 1985; Müller et al. 1989; Squartini and Sarnelli 1981; Tanaka and Oota 1970; Wellings and Jensen 1973) or correlation of serial subgross samples with radiographic examination (Egan 1982; Holland et al. 1985; Hutter and Kim 1971; Lagios 1977).

These studies, however, pose certain methodological problems. They all are prospective, time-consuming and often incompatible with routine diagnostic practice. In this paper we describe an original technique which allows the demonstration of the 3D aspect of a breast lesion from a conventional paraffin-embedded sample.

Materials and methods

Fifty-five tissue blocks were deparaffinized and analysed. This series consisted of samples from 32 mastectomy, lumpectomy or biopsy specimens. A high proportion of the patients (25) were operated on for malignant lesions. The 55 selected samples not only represented malignant tumours but also non-malignant breast lesions as well as normal breast tissue. The following histological structures and lesions were studied: cysts, fibrocystic disease, papillomas, adenosis (10 blocks), fibroadenomas (2 blocks) regressive and hyperplastic breast tissue (2 blocks), lobular carcinoma in situ and ductal carcinoma in situ (DCIS) (41 blocks). In cases of malignancy, the exact site from which the tissue blocks had been taken from the specimen as well as the thickness of the sample were known (4–5 mm). We are routinely examining such specimens with the correlated histological and radiological mapping techniques (Egan 1982; Holland et al. 1985).

The method for block preparation includes the following steps:

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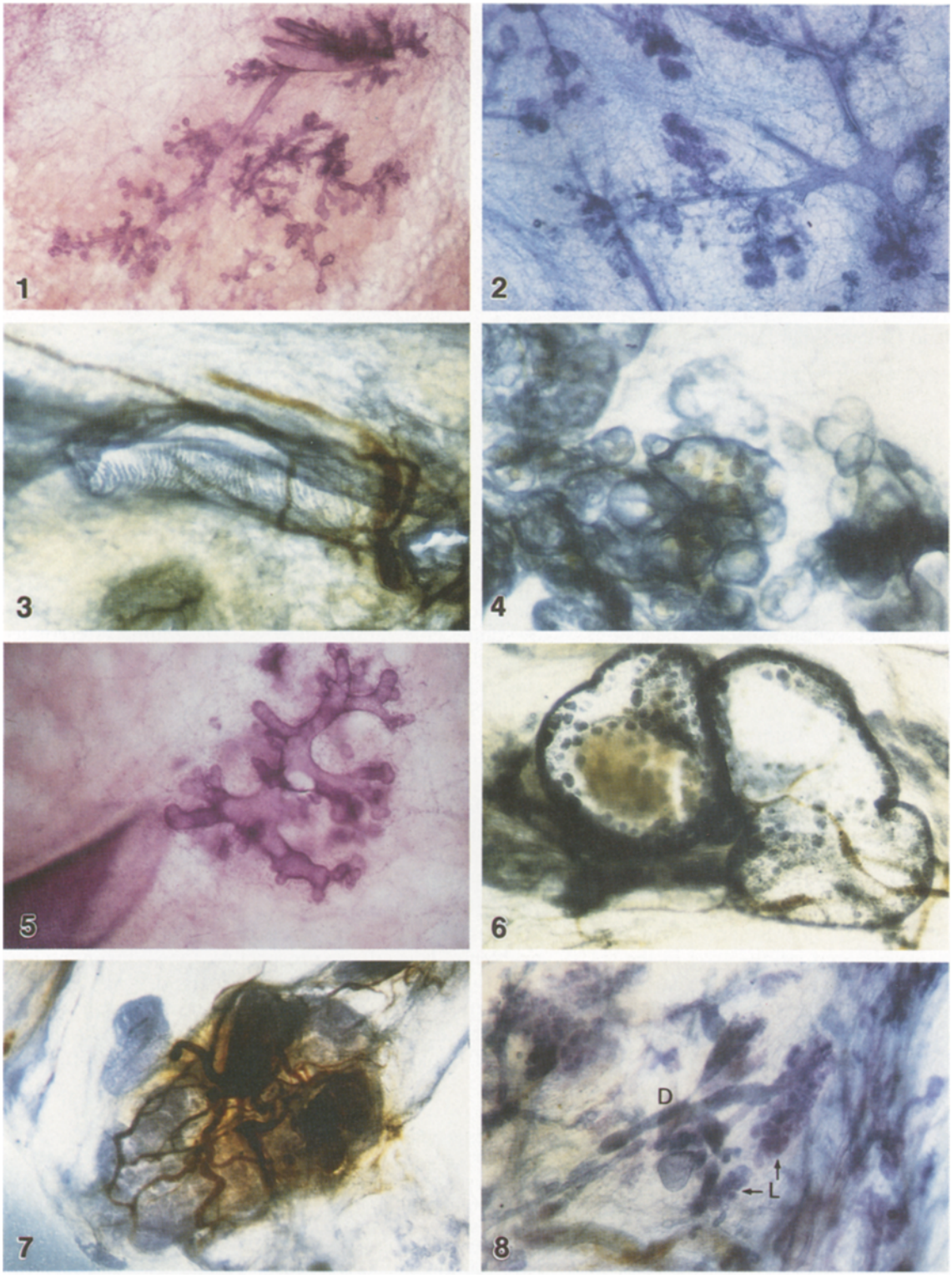


Figs. 1 and 2. Comparative stereoscopic views of normal breast tissue demonstrated with the presently described method (Fig. 1) and the Wellings' technique (Fig. 2). Mayer's haematoxylin, ×8

Fig. 3. Transverse striations in the wall of a duct with a micropapillary in situ carcinoma. Mayer's haematoxylin, ×20

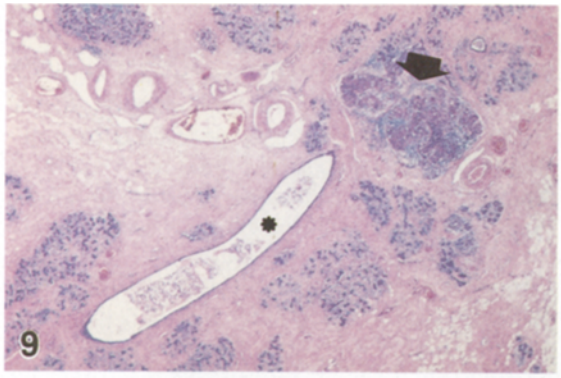
Fig. 4. Group of microcysts with psammomatous calcifications floating in the lumen. Mayer's haematoxylin, ×20

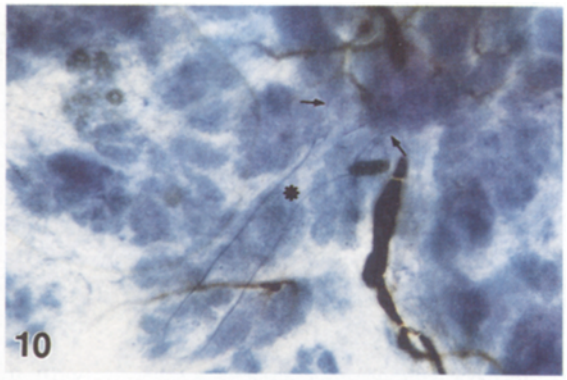
Fig. 5. Blunt duct adenosis of a terminal ductulo-lobular unit. Mayer's haematoxylin, ×30

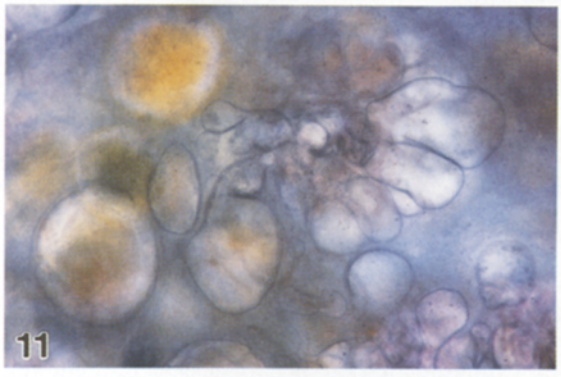
Fig. 6. Multilocular cyst lined with apocrine epithelium. Mayer's haematoxylin, ×12

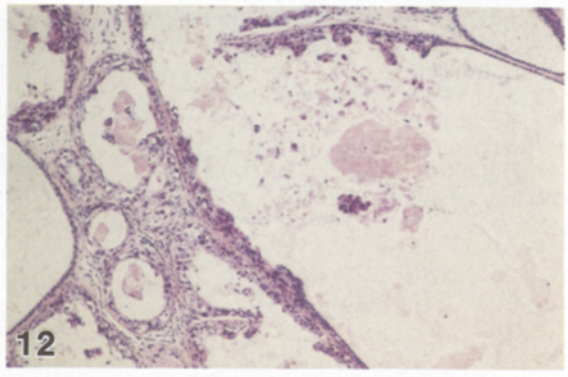
Fig. 7. Congested capillary network around a focus of ductal carcinoma in situ. Mayer's haematoxylin, ×10

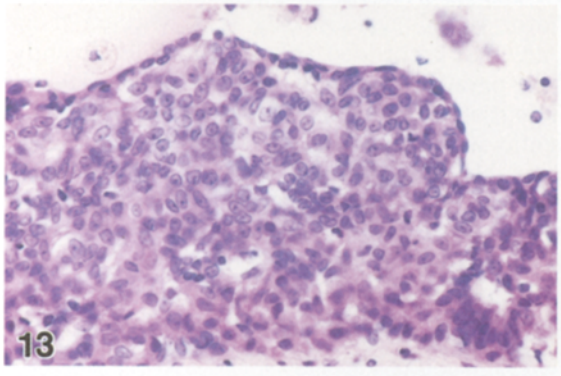
Fig. 8. This "flower-like" structure represent a terminal ductulo-lobular unit. The thickened part of the terminal duct (*D*) is hyperplastic and surrounded by two uninvolved lobular units (*L*). Mayer's haematoxylin, ×16

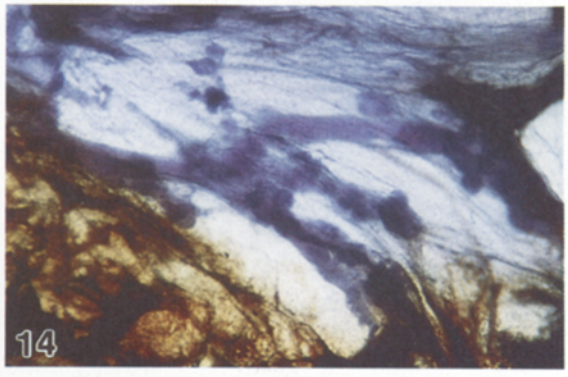


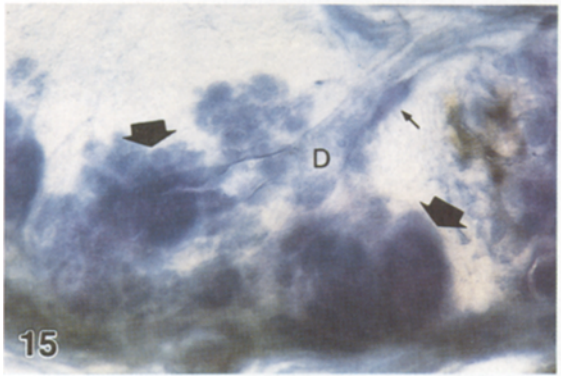


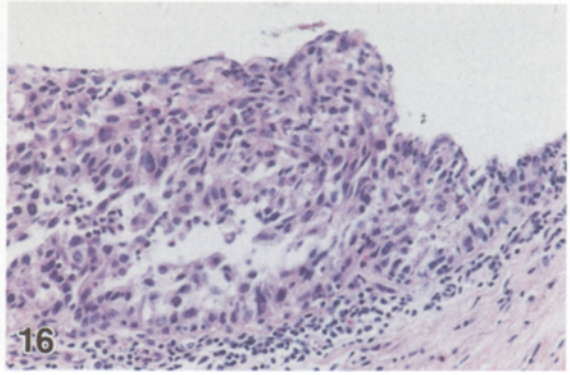












Figs. 9 and 10. This lobule (*thick arrow*) contains carcinomatous cells with pleomorphic nuclei (see Fig. 16) of the ductal phenotype. Nevertheless, the extralobular (*stars*) and terminal ducts (*thin arrows*) are not involved by the tumour, demonstrating the lobular origin of the neoplasm (**Fig. 9**, haematoxylin and eosin ×11; Fig. 10, Mayer's haematoxylin ×11)

Figs. 11 and 12. Stereoscopic and histological views of an intraductal carcinoma (clinging type). The 3D aspect is indistinguishable from microcysts. (Fig. 11, Mayer's haematoxylin ×6; Fig. 12, haematoxylin and eosin ×260)

Figs. 13 and 14. This example of small cell ductal carcinoma in situ shows a continuous growth pattern in the 3D ductal network. (Fig. 13, haematoxylin and eosin ×500; Fig. 14, Mayer's haematoxylin ×4.5)

Figs. 15 and 16. Illustration of the multifocal growth pattern of intraductal carcinoma. This terminal ductulo-lobular unit contains two foci of large-cell intraductal carcinoma (*thick arrows*) leaving a segment of the connecting duct (*D*) free from tumour. A separate focus of tumour is seen in a higher portion of the same duct (*thin arrow*). (Fig. 15, Mayer's haematoxylin ×11; Fig. 16, haematoxylin and eosin ×460)

1. Remelt the paraffin block.
2. Immerse the sample in successive xylol baths to remove the remaining paraffin
3. Rehydrate the tissue and rinse overnight in running tap water.
4. Stain for 10 min in Mayers' haematoxylin.
5. Wash in running tap water for 1 h.
6. De-stain in acid-alcohol for 1–2 h depending on the staining intensity required.
7. Wash overnight in running tap water.
8. Dehydrate 4–8 h in 96% ethanol.
9. Rinse 3 times in 100% ethanol for 36–48 h.
10. Put in acetone bath for 1–2 h.
11. Transfer to methylsalicylate for at least 3 h and seal in a polyethylene bag after which stereomicroscopic examination is possible.
12. For easier handling of the specimen, place the tissue on a standard microscope slide onto which a metal frame has been mounted with the following measurements: $24 \times 40 \times 3$ mm; fill the frame with DPX and place a coverglass over it.

Methylsalicylate impregnated tissue can be impregnated with DPX after rinsing in xylol and immersion in DPX for 48 h.

13. After 3D examination and photography, tissue specimens may be re-embedded in paraffin and processed for routine histology.

The complete procedure from deparaffinization to bag-sealing (steps 1–11) takes 5 days.

To compare the results of our method with that of Wellings, formalin-fixed samples of two cases from the series were stained according to both methods.

Results

The quality of the 3D image was very satisfactory (Fig. 1) and comparable with the results obtained with the subgross method of Wellings (Fig. 2). The best results were obtained with fatty breast tissue in which the duct structures are at some distance from each other, because under these conditions there was usually no superposition. However, the stereomicroscopic appearance of hyperplastic or proliferative breast lesions was still acceptable if the sample was not too thick (up to 3–4 mm) or fibrotic. The high definition of this technique permitted visualization of subtle details and tiny structures such as transverse striation in small cell micropapillary DCIS (Fig. 3), intracystic laminated calcifications (Fig. 4), blunt duct adenosis (Fig. 5) or apocrine metaplasia in fibrocystic disease (Fig. 6). Also of interest was the subgross aspect of the blood vessel network, which was directly visible due to the reddish-brown intravascular haemoglobin content. Capillaries seemed particulary congested in the close vicinity of some foci of DCIS (Fig. 7). In agreement with previous studies (Azzopardi 1979; Wellings and Jensen 1973), the majority of minimal benign and malignant breast lesions appeared to originate from the terminal duct or the TDLU. Conventional histology may already indicate the TDLU origin of some lesions but in other cases this may not be evident and can only be shown by stereomicroscopy. For instance, Fig. 8 shows the 3D aspect of a focus of ductal hyperplasia originating from a terminal duct; this would not be evident on examination of conventional haematoxylin and eosin-stained sections. In another case with a large-cell-type DCIS, an isolated malignant focus was discovered in a lobule with an intact terminal duct (Figs. 9, 10).

Some histologically proven malignant in situ lesions have a normal or non-specific aspect on stereomicroscopy. One case illustrates a DCIS (clinging type) in which the stereomicroscopic picture is indistinguishable from the normal galactophoric tree or from simple microcysts (Figs. 11, 12). It also shows the lobular arrangement of the process. The technique in addition allows to study the possible connections between foci of DCIS and their distribution and growth pattern in the ductal network. Some DCIS may grow continuously by extending through the glandular tree (Figs. 13, 14), but some of them may have a multifocal or stepwise progression with intact interposed ductal parts (Figs. 15, 16). This was observed in both large- and small-cell-type DCIS.

Discussion

The main purpose of the subgross techniques described in the literature has been to detect lesions and guide the sampling for histological examination. They represent valid methods to analyse multifocality in breast cancer (Hutter and Kim 1971; Müller et al. 1989; Wellings et al. 1975). Our technique is not a selection procedure; rather the sampling of the specimens is based on the gross and/or radiological aspects. The selected tissue samples are first embedded in paraffin. Subsequently, some of the paraffin blocks are chosen for the retrieval of the 3D structure on the basis of the examination of the conventional histological slide. The histological identification of the lesion precedes the stereoscopic examination and this allows 3D reconstruction of the breast lesions, the exploration of their precise location and their interrelationship within the mammary glandular tree. The 3D structure obtained from the paraffin block can show, for instance, whether two remote foci of DCIS in a histological slide are either connected by a neoplastic bridge or separated by an uninvolved ductal segment. To obtain a simple 3D picture of a DCIS process by using the previous techniques, the exploration of large pieces of breast tissue and the sampling of several apparently abnormal areas would have been necessary. Furthermore, as demonstrated in our study, some in situ malignant lesions observed in conventional histological sections, have a subgrossly normal appearance and were probably neglected in previous studies. This fact has been recognized by other authors (Wellings et al. 1975).

An approach similar to ours was developed by Ohuchi et al. (1984, 1985). They prepared serial sections from a paraffin-embedded sample containing the selected lesion and a 3D picture was reconstructed from the series of slides by using a graphic model. However, this procedure is rather labourious and fails to provide the in vivo aspect we are able to obtain directly from the paraffin block.

From a purely methodological point of view, our stereomicroscopic approach is probably one of the simplest. A routine pathology laboratory would be able to provide results equal to ours from their stock samples without the interference of its routine practice and without the

involvement of highly skilled technicians. As to the time involved, it requires in total some 5 h of labour and takes 5–7 days, whereas the Wellings' technique lasts more than 4 weeks (Wellings et al. 1975). Moreover, the use of DPX-framed slides allows easier handling and storing.

The 3D aspect of the galactophoric network can be clearly demonstrated and is very useful for teaching purposes illustrating breast pathology.

The method is also suitable for the exploration of both the origin and the distribution of DCIS. The latter has important clinical implications in breast-conserving surgical treatment, as the value of the assessment of the surgical margin is directly related to the growth pattern of DCIS, either continuous or multifocal.

Our study supports the Wellings concept that in situ carcinoma usually originates in the TDLU. Concerning the growth pattern of DCIS, Patchefsky et al. (1989) reported that multifocality varied from 42% to 68%, depending on the diagnostic procedures used. Ashikari et al. (1971) found the lesion to be multifocal in origin in 32% of their cases. In a recent study Holland et al. (1990) showed that DCIS of the large cell (comedo) type usually extend continuously through the ducts. In none of these studies were 3D techniques used.

We conclude that DCIS may grow continuously but can also have a genuine multifocal distribution with interposed preserved portions of ducts. In one case, for instance, an isolated focus of large-cell DCIS with pleomorphic nuclei was found inside a lobule, leaving the extralobular terminal duct free of tumour. As suggested by some authors (Azzopardi 1979; Wellings et al. 1975), an in situ malignancy with large cell phenotype may also develop within the lobule itself. The discovery of such a lesion in conventional histology should not be systematically regarded as a lobular involvement by a DCIS arising in extralobular ducts.

Our study indicates that there are, on the one hand, some rare exceptions of large-cell DCIS with multifocal distribution but that, on the other, small cell DCIS may also grow continuously. Combined with the Egan's serial subgross method, stereomicroscopic analysis will undoubtedly provide further information on the progression of DCIS.

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