

INTRA-OPERATIVE ASSESSMENT OF SENTINEL LYMPH NODES FOR BREAST CANCER SURGERY: AN UPDATE

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ABSTRACT

Lymph node (LN) involvement is the strongest prognostic factor in operable breast cancer (BC). Therefore, accurate assessment of LN status is essential for management of BC patients. The introduction of sentinel LN approach reduced the need for extensive axillary surgery to achieve accurate staging. However, positive sentinel LN as determined on postoperative histological examination often leads to a second axillary operation to ensure an accurate staging and that positive non-sentinel LNs are removed. Although preoperative assessment of LN has improved significantly, its accuracy remains insufficient to avoid further axillary surgery and is not sufficient to predict the status of the LN. Therefore, intraoperative evaluation of the sentinel LN to determine the need for completing lymph node dissection in case of metastasis can provide an important approach to guide BC management decision making. This article reviews the techniques available and under development for intraoperative detection of sentinel LN metastasis in BC surgery. The key features of each technique are described in detail, emphasising the benefits offered by label-free optical techniques: minimal sample preparation, high spatial resolution, and immediate on-site implementation. Optical techniques have the potential to provide a cost-effective and accurate intraoperative platform for the assessment of SLN within the operating theatre.

KEYWORDS

Intra-operative assessment; lymph nodes; Breast Cancer; Raman spectroscopy

INTRODUCTION

Breast cancer (BC) is the most common cancer in women (1). There are about 55,000 new cases diagnosed annually in the UK and 2.2 million new cases globally (1). Axillary lymph node (LN) status is the most important prognostic factor in operable BC. Therefore, all operable BC patients undergo axillary LN sampling to accurately stage the disease and direct treatment decision making (2, 3). When a breast tumour metastasizes, the sentinel lymph nodes (SLN) are the first ones to be involved (4). Patients with a preoperative diagnosis of axillary nodal metastasis often proceed to axillary lymph node clearance (ALNC) during BC surgery (5, 6). Negative SLN, as demonstrated by the preoperative imaging and the postoperative histological examination, eliminates the need for a second axillary operation. Therefore, assessment of SLN status is crucial for treatment of the axilla and to avoid the side effects of unnecessary ALNC (7, 8). Although there is a shift in many countries towards a conservative axillary approach to avoid ALNC in cases with axillary metastases confined to 1–2 SLNs (9), this practice is not implemented in all centres. Furthermore, it is not applicable to patients not fulfilling the criteria of the trials demonstrating this finding (ACOSOG Z0011, IBCSG 23-01 and AMAROS), including patient age, receptor status and the plan to offer local and systemic therapy in addition to patients who were offered neoadjuvant treatment or planned for mastectomy (10-12). In the ACOSOG Z0011 trial the median number of sampled SLN was 2 (range 1-4) (11). There is also evidence that approximately a third of patients who undergo ALNC had additional positive nodes (13) and if the patients are not receiving an effective therapy and the outcome can be poor particularly in the era of de-escalation of systemic therapy options. Therefore, ALNC or extended axillary node sampling (14) is expected to continue as the standard of care for positive SLN at least in some countries.

Several techniques have been proposed for the assessment of SLN in BC patients. These techniques can be broadly divided into three categories – pre-operative, intra-operative and

post-operative methods (*Supplementary material, Figure S1*). The pre-operative identification of nodal metastasis has clear advantages because the SLN biopsy is not performed on node positive patients and ALNC is performed directly during the primary BC surgery (3, 15). Clinical variables such as tumour size, grade and lymphovascular invasion have some predictive value of LN metastasis; however, no combination of predictors of axillary LN has replaced histological examination of the LN (16). While advances in ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) can often identify suspicious nodes in the axilla, false-negative findings, and failure to detect small metastases are common and the cost of some of these techniques is often prohibitive. There is evidence that preoperative image-based axillary staging has a median utility in only 20% of BC patients who had axillary surgery after a positive needle biopsy (17). The post-operative techniques include histopathology with or without immunohistochemistry are considered as the gold standard and practised routinely. However, histology is time consuming, and when SLN are proven positive on histopathological examination (around 20% of cases), a second axillary surgery is typically performed (18). Therefore, intraoperative assessment of SLN can help to determine the immediate definitive axillary surgery without the need for further surgery in SLN positive cases confirmed by postoperative histopathological examination (18). In this article, we discuss the various techniques used to assess SLN status intra-operatively in detail.

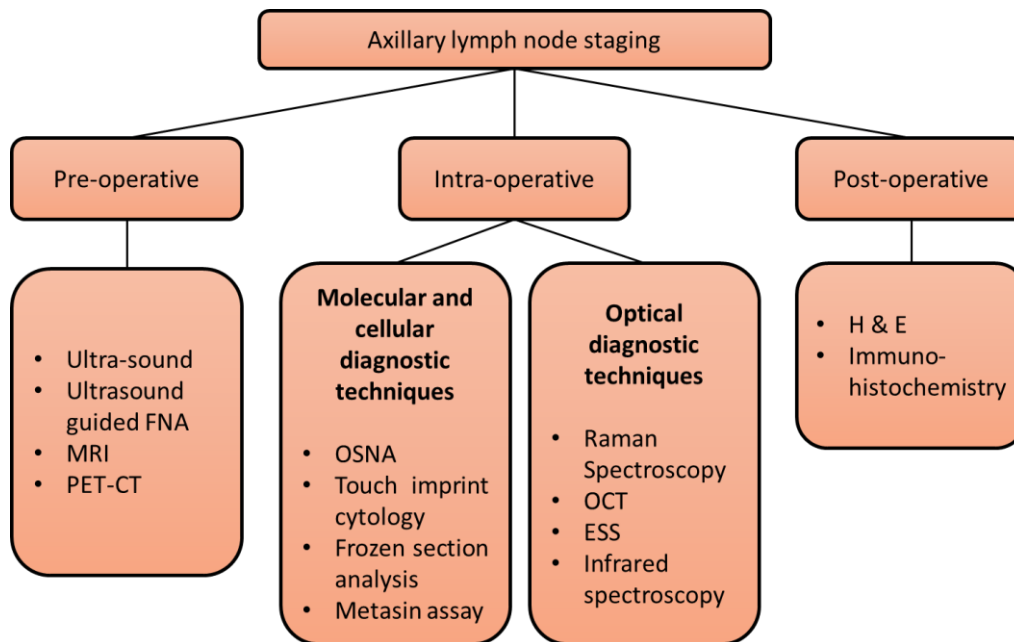


Figure 1: Various techniques used in axillary lymph node staging listed as pre-operative, intra-operative and post-operative methods (FNA: Fine Needle Aspiration; MRI: Magnetic Resonance Imaging; PET-CT: Positron Emission Tomography – Computed Tomography; OSNA: One Step Nucleic-acid Amplification; OCT: Optical Coherence Tomography; ESS: Elastic Scattering Spectroscopy).

METHODS OF INTRA-OPERATIVE ASSESSMENT OF SLN

An ideal intra-operative technique for SLN assessment should: 1) provide results within the intra-operative time duration, 2) have high sensitivity and specificity, 3) be easy to use and not requiring specialised expertise, 4) not consume sample so the results can be verified by histopathology and 5) be cost-effective. Although several techniques are applied for the intraoperative assessment of SLN to achieve these aims, most of the existing techniques have limitation and no consensus on one method has been achieved. Some of these techniques are traditional and used for a long time (such as imprint cytology, evaluation of cells scraped from the cut surface of the node, or frozen section examination), but some are recently developed (such as detection of epithelial markers in the SNL fresh material) and some are

emerging (such as spectroscopic techniques). Here we describe the most common intraoperative methods with emphasis on the recent and emerging techniques.

1- CYTOPATHOLOGICAL EXAMINATION

A-Frozen section examination

The protocol for frozen section analysis is explained in detail by Turner et al. (19), which involves slicing the fresh LNs, stain tissue sections with H&E and microscopic review and interpretation of H&E stained slides by a pathologist. This is the first and most commonly used methods in many centres and it is associated with a reasonable degree of accuracy. Sensitivity and diagnostic accuracy of frozen sections range from 55-83% and 90-100%, respectively. Sensitivity is significantly higher in finding macrometastases compared to micrometastases (20-22). A meta-analysis of 13,000 patients demonstrated an overall sensitivity of 73%, which was reduced to 40% in micrometastasis though the mean specificity was 100% (23).

Although the LN frozen section analysis is associated with high specificity and reasonable sensitivity and can be performed in intra-operative time duration, there are certain disadvantages. These include sampling limitations, technical problems in preparing the slides and interpretative error in addition to the need for expert pathologists to be available to read the slides intraoperatively, which is a major concern given the shortage of pathologists worldwide (24, 25). The quality of frozen tissue preparations is seldom as good as those prepared from well-fixed tissue, and upon freezing may compromise the quality of paraffin section histology. The technical problems associated with frozen section analysis also include freezing artefacts, which cause damage to the tissue structure and poor morphology that can affect the reading of the slides (26).

B- Touch imprint cytology

The procedure of imprint cytology is described by D. W. Chicken et al. (27). The sentinel nodes were initially bisected along the long axis. Positively charged microscopy slides were used for imprinting the bisected node. Multiple imprints were taken from each section of the lymph nodes before air-drying. After staining the slides using May-Gruwald-Giemsa stain, the slides were examined by a pathologist. Similar to frozen sections, touch imprint cytology has a sensitivity and specificity of 50-90% and 99-100% respectively (27-29). In one study, the intraoperative examination of SLNs from 413 women with BC was carried out using imprint cytology (30). Upon comparison with H&E and immunostaining for cytokeratin, sensitivity was found to be only 36% whereas overall accuracy was 99%. In a meta-analysis of imprint cytology for assessing SLN metastasis in breast cancer, which included 31 studies, a pooled sensitivity and specificity was found to be 63% (CI 57%-69%) and 99% (CI 98%-99%). Pooled sensitivity for macro-metastasis was 81%, which was reduced to 22% in micro-metastasis.

The major advantage of imprint cytology is that it does not require developed infrastructure (31), gives immediate results with minimal artefacts and is inexpensive. The disadvantages include lack of depth information and inability to differentiate micro-metastasis from macro-metastasis or even a metastasis from BC from that of other cancers (32, 33). The majority of the false negative cases were associated with micro-metastasis and sampling errors. The technique demands a cytologist to be present on sight and is not used in most of the centres due to the impracticality of providing the expertise during surgery.

2- MOLECULAR TECHNIQUES

These techniques are based on the identification of tumour cell specific molecular markers with the commonly used epithelial cell markers including low molecular weight cytokeratins. These markers are expressed by tumour cells but not by lymphoid cells. Therefore, their detection using any suitable assay indicates a positive lymph node. The most commonly used molecular tests for intraoperative assessment of SLN are one step nucleic acid amplification (OSNA) and Metasin tests.

A- One step nucleic acid amplification (OSNA)

Tsujimoto et al. (34) reported the development of one step nucleic acid amplification (OSNA) assay for detecting LN metastasis using 325 LNs from 101 patients. The overall sensitivity was 98.2% without reported false positive results. The test is based on the detection of cytokeratin-19 (CK19) that is frequently expressed by BC cells. It involves homogenisation of LNs and analysis using RD-100i system with reverse transcription loop-mediated isothermal amplification (RT-LAMP) method (34). The main advantages of OSNA are the high sensitivity, analysis time compatible with intra-operative-use, minimum supervision or being able to use the method without a molecular biology expert. In a meta-analysis of OSNA for LN assessment in breast cancer patients that included 5057 lymph nodes from 2192 patients extracted from 12 studies (35) the sensitivity and specificity were 87% (CI 81%-93%) and 98% (CI 96%-100%) respectively. In a similar meta-analysis that included 2833 patients (36), the pooled sensitivity and specificity were 87% (CI 81% - 91%) and 92% (CI 86% - 95%) respectively. The National Institute for Health and Care Excellence (NICE) has endorsed OSNA for the use in the intraoperative detection of LN in 2013 (37).

The time required for OSNA diagnosis is about 30-45 min (38, 39). It was claimed that the test can be performed without molecular biology expert (40) and unlike frozen section or touch imprint cytology it does not require staining of tissue. However, as the test utilises the

LN tissue it is difficult to exclude the possibility of false positive cases in the processed tissue. Intraoperative evaluation of SLNs via OSNA technique was performed in some centres in UK (38). Discordant cases were analysed again by both methods to determine whether the cause was due to tissue allocation bias. After exclusion of samples with tissue allocation bias, the sensitivity and specificity obtained was 92% and 97% respectively. The consumption of the nodal tissue makes the test a black box and further confirmation of the results using the gold standard histopathological examination not possible. Because both OSNA and histology cannot be performed on the same sample, the OSNA test can be performed on half the node or alternative node, which allows follow up histopathology, but increases the risk of tissue allocation bias. Due to this reason, it is recommended by NICE to implement the OSNA test of the whole LN. However, this can be considered as another disadvantage of OSNA not being able to perform histological confirmation for validation of the technique. In addition, some cases the tumours do not express CK-19 (1-7%) (41, 42). Therefore, OSNA tests may result in false negative cases. NICE recommends pre-screened CK19 gene expression of tumour biopsy sample and if there is no CK-19 gene expression the OSNA should not be used for LN assessment. But this will result in using limited pathology resources and not being able to produce results during surgery. Moreover, due to tissue heterogeneity, the interpretation of results may be complicated. Furthermore, the test can be positive in the rare cases of metastatic carcinoma other than breast and cases of intranodal inclusion by epithelial elements.

B- Metasin test

Metasin test is a molecular test similar to OSNA but uses the quantitative reverse transcriptase PCR (qRT-PCR) to detect metastasis markers –CK19 and mammaglobin. The breast epithelial cells and BC cells express a high level of mammaglobin. The results can be obtained in 32-36 minutes, including 6-10 minutes required for extracting and purifying

mRNA (43). In a study by Giridhar P. et al. (44), Metasin test was performed on RNA samples from 3296 SLNs from 1836 patients. Alternate tissue slices were examined by histology. The sensitivity and specificity obtained were 92% and 97%, respectively. The positive predictive value was 88% and half the conflicting cases were attributed to tissue allocation bias. Similar results were also obtained in a study including 250 patients with 533 SLNs (45). One additional limitation of the Metasin test is that the mammaglobin marker is not expressed in all breast tumours and hence patients require pre-screening of mRNA from tumour biopsies. The proportion of cases that does not express mammaglobin is not known. Similar to OSNA, there is also tissue allocation bias and not enough studies about the discordant analysis.

3- OPTICAL TECHNIQUES

A variety of optical spectroscopy techniques, such as Raman spectroscopy, fluorescence spectroscopy, elastic scattering spectroscopy (ESS), Fourier transform infra-red (FTIR) spectroscopy have been explored in a broad range of pathologies. In general, optical techniques require minimal or no sample preparation, have high spatial resolution and potential for on-site implementation. Some optical techniques can provide quantitative diagnosis, therefore may reduce diagnosis subjectivity and the need for specialist users.

A- Elastic scattering spectroscopy

In ESS, light scattered elastically by tissue is collected via an optical probe, which is passed through the working channel of an endoscope, such that it comes in direct contact with the tissue (46). Results can be obtained within fractions of seconds, after short pulses of white light probe the tissue. In the field of cancer, ESS has been used to examine 139 LNs from 68 patients, using an instrument with a pulsed broadband light source (47). Principal component

analysis and linear discriminant analysis of the spectra were performed before comparing them with conventional histology results. The ESS resulted in a sensitivity and specificity of 75% and 89% respectively. Similar results have been obtained by other studies (48, 49).

The main advantages of the ESS technique are the simple and inexpensive set-up, instant results, minimum sample preparation. The technique can be automated and hence can be performed without an expert. The disadvantage of ESS for diagnosing metastasis is its low sensitivity and specificity.

B- Optical coherence tomography

Optical coherence tomography (OCT) is an optical imaging technique that measures the time delay and amplitude of backscattered light (50). The working principle of OCT involves using a low coherence light beam as the source and backscattered light from tissue is combined with a reference beam using interferometry. The interference patterns are reconstructed along the beam path, which captures the tissue scattering properties. By changing the incident point on the tissue in a line, a two-dimensional image of the tissue can be reconstructed. Detection of metastases in tissue requires a trained user to identify specific features in the OCT images.

The first report of OCT applied for LNs is published in 2005 by the Boppart group at the University of Illinois (51). OCT images enabled visualisation of LN structure including micro-metastasis proving the potential of the technique to be used intraoperatively (52). A total of 314 specimens from 173 patients including 141 LNs and 173 breast biopsies were used for OCT imaging and resulted in 66.7% sensitivity and 79.6% specificity compared to histology results. Intraoperative assessment of LNs using 3D OCT was reported by Nolan et al. (53). A total of 184 images from 128 LNs were included in the study. The overall sensitivity and specificity obtained was 58.8% and 81.4% respectively. The false negative

cases were attributed to image artefacts and reader analysis difficulty. Figure 2 shows an example of how OCT images can be correlated to H&E images. Variations in scattering are used for differentiating between healthy and metastatic LNs.

Key advantages of OCT are the ability to provide quick results (nearly 25s for a full volumetric block of images of size 5x5mm²) compared to traditional diagnostic techniques, provide high resolution images and is a portable device (54). One disadvantage though is the use of femtosecond lasers that are expensive and the penetration depth is limited by the opacity of samples. Excess adipose tissue surrounding the LNs can obstruct OCT signal and LN tissue can be absent from the tissue. Also, highly dense stromal tissue can produce a strong OCT signal and can shadow a cancerous region. Moreover, the fact that metastasis needs to be assessed based on the morphology of the LNs in the OCT images, implies extensive training of the staff and results can be subjective.

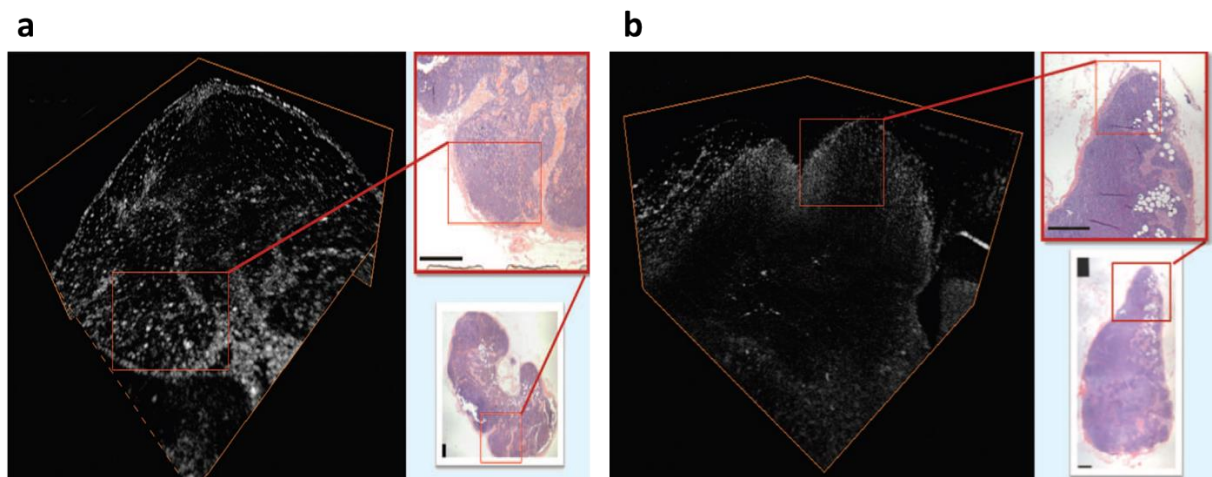


Figure 2 : (a). The normal lymph node shows a clear capsule that is easily differentiated from the low-scattering cortex. The OCT images correlate to the regions in the histology that are highlighted by the red boxes. (b) Three-dimensional rendering of a metastatic lymph node. OCT image volume acquired from a metastatic axillary lymph node in a single session is shown with the corresponding H&E histology. Increased scattering from the node is observed in the OCT data, and the ability to differentiate distinct boundaries between the node capsule and cortex has been lost. The OCT images correlate to the regions in the histology that are highlighted by the red boxes. The OCT image block measures 53 531.7 mm³. (Reproduced from (55))

C- Infrared spectroscopy

In infrared (IR) spectroscopy, the absorbance of electromagnetic waves at infrared wavelengths is measured to determine the molecular structure and composition of tissue. The IR light transmitted or reflected from the sample is detected after passing through the interferometers, and the IR spectra can be computed based on the Fourier transform of the interference pattern.

Intra-operative application of Fourier transform infrared spectroscopy (FTIR) was explored for detecting lymph metastasis (56). A total of 149 fresh LNs from 49 patients with BC was used for the study. When compared with histological results the technique achieved 94.7% sensitivity and 90.1% specificity. Although 95% confidence intervals were not reported, their estimates are 82-99% for sensitivity and 84-95% for specificity (Clopper-Pearson intervals). Infra-red spectral imaging was able to detect micro-metastasis in LNs (57), which was enabled by a high spatial resolution of 10-12 μ m. In these studies, tissue sections were cut into 5 μ m sections, de-paraffinized and dried without staining. Data acquisition from 1mm² image took about 40 minutes. Figure 3 shows a cluster image of LN constructed from FTIR spectra compared to the H&E image and the micro-metastasis region can be clearly identified. Infrared spectroscopic image from LNs was able to identify different types of cells such as memory B cells, T cells, erythrocytes, fibro-vascular network etc.

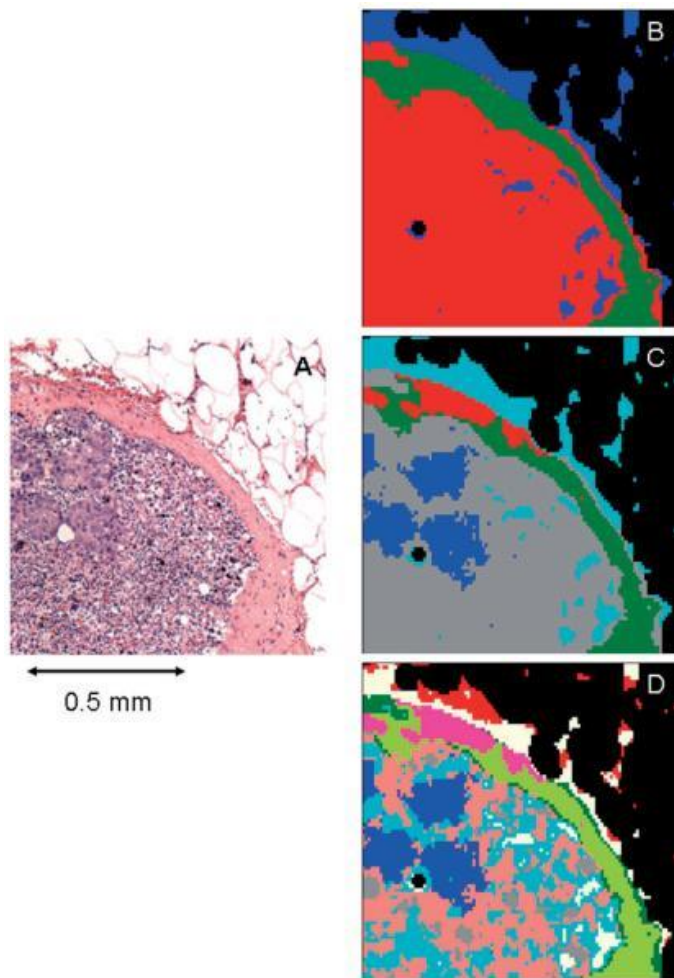


Figure 3: (A) Microscopic image of lymph node tissue section (B) 3-cluster spectra image constructed in 900-1800 cm^{-1} region (C) 5-cluster image obtained from the same region (D) 9-cluster image obtained from the same data set. The micro-metastasis region - marked in Blue colour - is apparent in images A, B and C (Reproduced from [51])

FTIR is an accurate, non-destructive and cost-effective technique (58). The reports of infrared spectral imaging techniques have been focussed on LN structure details and there are very few reports on intra-operative detection of LN metastasis. Because water absorbs strongly infrared radiation, measurements on fresh tissue have typical large variations, and achieving high diagnosis accuracy requires extensive drying. Despite promising results being published in 2009 [51], we are not aware of instruments being used/tested in the clinic for intra-operative SLN diagnosis.

D- Fluorescence imaging

In fluorescence imaging, a light source, usually laser is shone on the sample of interest and fluorescence light is detected to construct an image. The variations in the molecular structure of normal and metastatic cells can enable the differentiation of the two. Rigacci et al. (59) used micro-spectrofluorometry and autofluorescence imaging technique for analysing LNs from patients with lymphadenopathy. 365 nm excitation wavelength was used for getting tissue autofluorescence images from LN sections. Monochrome images were obtained using interference filters at 450 nm, 550 nm and 658 nm and combined to make a single red-green-blue colour image. Morphological differences were observed in normal and neoplastic tissues. A loss of follicle organisation was observed in neoplastic tissues and weakly fluorescing follicles separated by connective trabeculae, which were highly fluorescent. This is a qualitative study that involved LNs from five patients. In a fluorescence imaging study of LNs from head and neck cancer patients a contrast agent, panitumumab-IRDye800CW (injected 2 days before the surgery) was used for identifying metastasis (60). With a total 1012 LNs from 24 patients with 39 metastatic LNs (average 37.5 LNs per neck with range 12-72 nodes), sensitivity and specificity of 84.6% and 94% was achieved with positive predictive value and negative predictive value of 36.2% and 99.3% respectively.

The disadvantage of autofluorescence imaging is low specificity. Fluorescence imaging has been reported more frequently for fluorescence guided biopsy rather than intra-operative detection of metastasis.

E- Raman spectroscopy

Raman spectroscopy is an alternative technique for measuring the vibrational spectrum of a sample. Raman spectroscopy measures inelastically scattered photons when the molecules in the sample are excited by a laser. The differences between the Raman spectra of normal and

metastatic nodes, often quantified using multivariate statistical models, can then be used for detecting metastasis. Smith et al. (61) showed that the presence of different molecules can be mapped across LN sections using Raman spectroscopy. The identification of nucleic acids, proteins, carotenoids, and lipids via Raman spectroscopy suggested the possibility of comparing these constituents to understand the biochemistry involved in breast carcinoma.

Smith et al. (62) investigated the use of Raman spectroscopy for axillary LN classification. The study involving 59 LNs from 58 patients and reported 91% sensitivity and 93% specificity. Various investigations aimed at applying Raman spectroscopy for intra-operative assessment of LNs and the overall sensitivity varied between 80% to 90% and specificity between 85% to 100% (63-65). Figure 4 shows the Raman spectroscopy evaluation of LNs.

Raman spectroscopy has several advantages over other spectroscopy techniques such as no sample preparation, non-destructive, quantitative, higher sensitivity, and specificity etc. These reasons make Raman spectroscopy a potential candidate for intraoperative assessment of LNs.

COMPARISON OF THE METHODS

Table 1 lists the intra-operative techniques used for the assessment of lymph nodes, their advantages, and disadvantages.

Table 1: Intra-operative techniques used for SLN assessment and their advantages and disadvantages

Technique	Advantages	Disadvantages	Reference
Frozen section analysis	Takes around 20-30 min; high specificity, close to 100%	Freezing the sample creates significant artefacts and tissue loss and hence the sample may not be suitable for further histopathological assessment; low sensitivity: 44%-100%	(24-26, 66)
Touch imprint cytology	Does not require developed infrastructure; immediate	Lower sensitivity (80%-96%); need for cytopathology	

	results with minimal artefacts; inexpensive	expertise	(31-33)
One step nucleic acid amplification	30-45 min for results; high sensitivity, intra-operative time duration, minimum supervision or being able to use the method without molecular biology expert and importantly the ability to detect micro-metastasis more frequently compared to routine histology.	Tissue allocation bias upon using half LN; recommended to use the whole node but will not allow follow up histopathology; CK-19 not expressed in all cases resulting in false negative cases	(38, 39, 41, 42) (67)
Metasin	Reported to take around 26 minutes after 6-10 min for mRNA extraction and purification	Molecular biology expertise needed which can result in loss of pathology expert in the laboratory; concerns regarding maintaining quality during RNA extraction in the operating theatre; mammaglobin not expressed in all cases	(43, 44)
Optical Techniques			
Raman spectroscopy	High specificity and sensitivity, does not consume sample, minimum sample preparation	Long scanning time	(68)
Optical coherence tomography	Tissue cross-sectioning with high resolution, quick results, computerised, portable device	Low sensitivity, Requires trained user to recognise metastases in OCT images	(51, 54)
Elastic scattering spectroscopy	Results within a short period of time, high specificity, minimum sample preparation, simple and inexpensive set-up	Low sensitivity, using ultra-violet radiation	(46, 47)
Fourier transform infrared spectroscopy	Detailed information on LN structure, high sensitivity, and specificity.	Interference due to presence of water which absorbs IR radiation; total time required for sample preparation and spectral acquisition can take more than an hour	(56, 58)

Table 1 indicates that none of the optical techniques currently fulfil all the requirements for intra-operative assessment of SLNs. As most of the clinically available intraoperative examination techniques have limitations, their use for assessment of the SLNs is decreasing. For instance, a UK survey of pathological evaluation of staging ALNs found that only 10% of labs used frozen section or imprint cytology (69). A 2012 study from the Memorial Sloan Kettering Cancer Center, USA found that the use of SLN frozen sections decreased from 100% to 62% over a 10-year period (70). Therefore, novel intraoperative examination techniques that are clinically available, feasible, accurate and cost efficient are likely to encourage the use of this examination approach and return to higher rates of intraoperative examination of SLN to further reduce the rate of completion ALNC.

While Raman spectroscopy has high diagnostic accuracy and provides the prospect of quantitative diagnosis (no need of trained user), acquiring spectra from whole tissue samples can take several hours. Raman spectroscopy is molecular specific and hence heterogeneous samples like tissue, which has complex molecular structure and distribution require the region from where the bio-signals are acquired needs to be recognised. So, combining Raman spectroscopy with other techniques, such as fluorescence imaging or OCT may be a solution.

The integration of Raman microscopy and fluorescence imaging was proposed for intra-operative detection of positive margins in non-melanoma skin cancer surgery (71, 72). The technique combines auto-fluorescence imaging, which is fast, has high sensitivity but low specificity) with Raman spectroscopy, which can attain high specificity and sensitivity but has a low speed. Automated segmentation of auto-fluorescence was used to select region for Raman spectroscopy. The diagnosis which is based on the spectral classification model allowed a 100% sensitivity and 92% specificity in diagnosing basal cell carcinoma in skin tissue samples. Prototypes based on this technology were developed to allow use by clinical staff (73), and are currently being integrated in the clinic (74).

Shipp et al. (75) used a similar multimodal spectral histopathology (MSH) approach to identify residual tumour at the excised breast tissue surface during breast conserving surgery. Raman spectra were recorded from a smaller region of the sample based on the autofluorescence image. 121 samples from 107 patients including 51 fresh, whole excised specimens were used for the study which gave 95% sensitivity and 82% specificity. The study proved the potential of MSH in intra-operative applications by reducing the time to 12-24 min. Figure 4 shows MSH diagnosis of breast tissue produced using combined autofluorescence imaging and Raman spectroscopy. While MSH agrees with H&E results, it has also reduced the overall acquisition time compared to raster scanning.

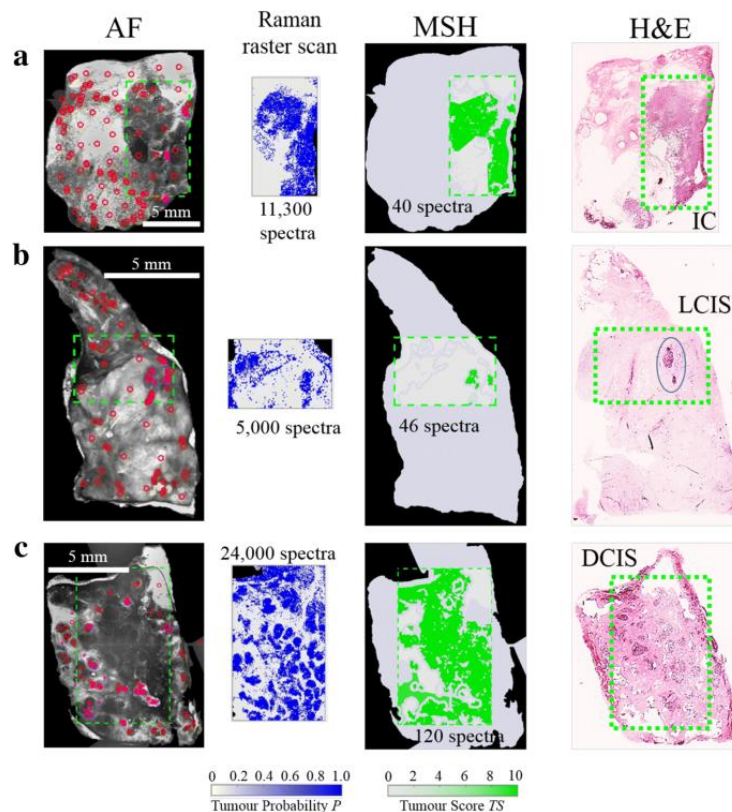


Figure 4: Multi-modal spectral histopathology (MSH) diagnosis generated using autofluorescence (AF) and raster scan Raman measurements of breast samples. Diagnosis for Raman raster scan is presented as tumour probability (P) (output of the classification model), while the diagnosis of each segment in the MSH is presented as tumour score (TS). Segmentation and sampling algorithms use AF images to focus Raman measurements (red circles) to suspicious regions, greatly reducing the number of spectra required for accurate diagnosis. Areas detected as a tumour in the first round of MSH measurements are sampled

by further Raman measurements (magenta crosses). a) Invasive carcinoma (IC); b) lobular carcinoma in situ (LCIS); c) ductal carcinoma in situ (DCIS) (Reproduced from (75)).

The techniques Raman spectroscopy and autofluorescence has the advantage of not requiring external stain, can give real time results and provide information on the biological structure organisation. Multispectral imaging technique may provide more accurate results in less time making it suitable for intra-operative assessment of SLN.

CONCLUSIONS

Various techniques have been reported for intra-operative assessment of LN metastasis. Pathological techniques like frozen section analysis and touch imprint cytology can be performed within the intra-operative time duration. But both techniques have lower sensitivity and require the availability of pathologists. Although molecular techniques such as OSNA and Metasin have shown potential to be used intra-operatively, these techniques consume the node tissue and do not distinguish micro from macro metastatic disease. Optical techniques like OCT and ESS can give results within minutes and require minimum sample preparation. However, the ESS has low sensitivity and interpreting OCT images needs extensive training. The autofluorescence technique can be quick and have higher sensitivity but it has low specificity and has been mostly used for SLN biopsy surgery guiding. On the other hand, vibrational spectroscopy techniques such as IR and Raman spectroscopy can provide similar or better performance without using the tissue or requiring contrast-enhancing agents. IR technique needs the LNs to be sliced into thin sections followed by drying to eliminate water which can lead to additional laboratory infrastructure, personnel, and time. In contrast, Raman spectroscopy has no interference from water, and it allows verification by histological results. The longer acquisition time required by Raman spectroscopy can be

overcome by combining with other techniques like autofluorescence imaging. This will not only speed up the assessment but also results in higher sensitivity and reduces the overall detection time making it an ideal candidate for intra-operative diagnosis. Most of the molecular and optical techniques described above are sensitive and detect low volume metastatic disease such as micrometastases. Although in many settings and practices micrometastases would not automatically mandate ALNC, micrometastatic disease can lead to decision to perform ALNC in certain scenarios such as the post neoadjuvant treatment setting. Importantly Raman spectroscopy provides a map of the node and the metastatic tumour outlines therefore, the size of the metastatic deposits can be recognised and decision to proceed to ALNC, sample more nodes (lymph node sampling) or not can be made based on the information provided and the clinical context.

LIST OF ABBREVIATIONS

ALNC – Axillary Lymph Node Clearance

ANN: Artificial Neural Networks

BC: Breast Cancer

CI: Confidence Intervals

ESS: Elastic Scattering Spectroscopy

FNA: Fine Needle Aspiration

FTIR: Fourier Transform Infrared

IR: Infrared

LDA: Linear Discriminant Analysis

MRI: Magnetic Resonance Imaging

MSH: Multi-modal Spectral Histopathology

NICE: National Institute for Health and Care Excellence

OCT: Optical Coherence Tomography

OSNA: One Step Nucleic Acid Amplification

PCA: Principal Component Analysis

PCR: Polymerase Chain reaction

PET-CT: Positron Emission Tomography-Computed Tomography

SLN: Sentinel Lymph Node

SLNB: Sentinel Lymph Node Biopsy

SVM: Support Vector Machines

DECLARATION

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Authors' contribution

ER contributed to the idea and structure of the review, reviewed, and approved the manuscript. IN contributed to the overall plan of the paper and supervised the description of the optical technique. SB carried out the literature survey with inputs and guidance from IN and ER.

Competing interests

The authors declare that they have no competing interests

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