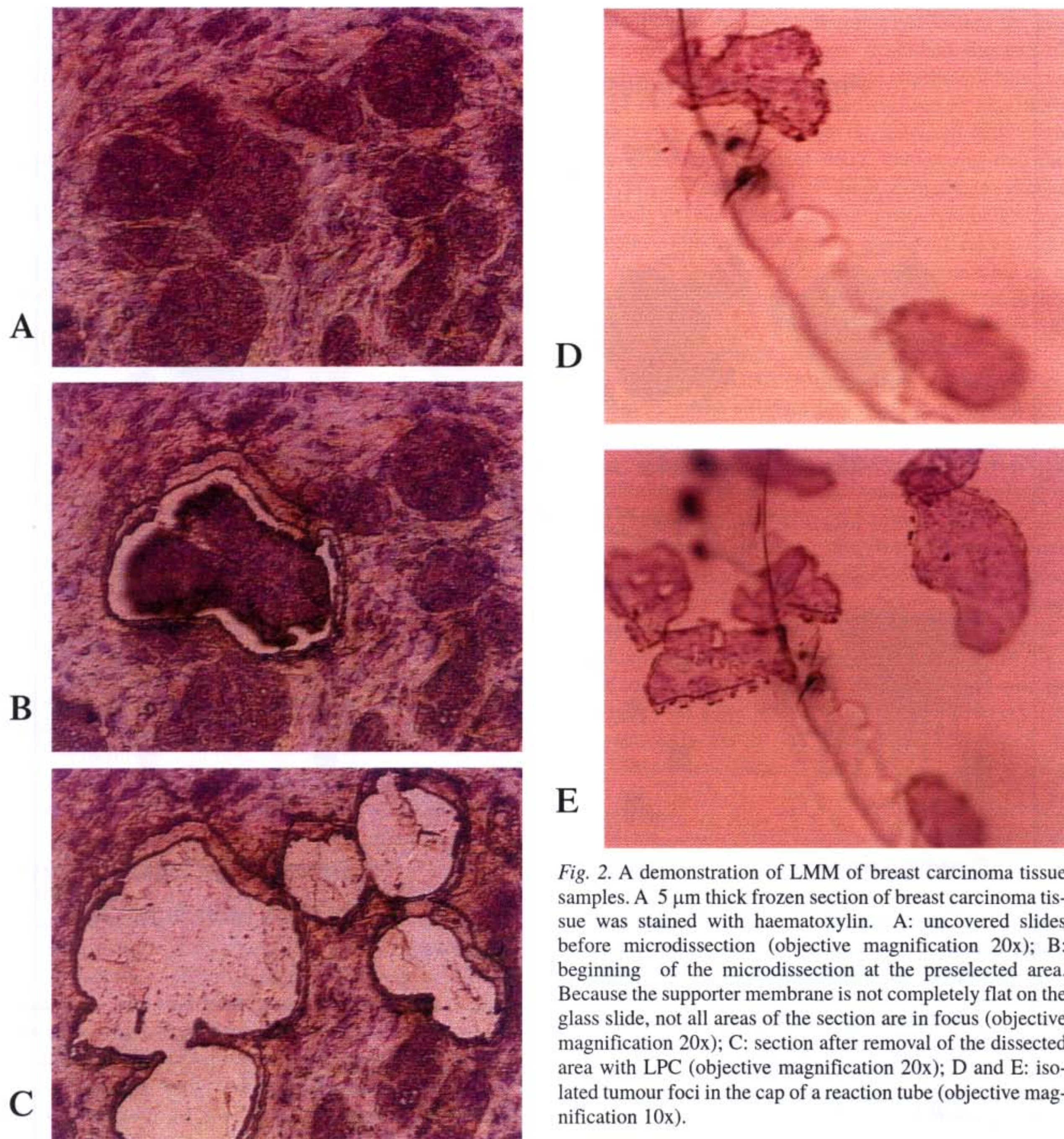

Fig. 2. A demonstration of LMM of breast carcinoma tissue samples. A 5 μm thick frozen section of breast carcinoma tissue was stained with haematoxylin. A: uncovered slides before microdissection (objective magnification 20x); B: beginning of the microdissection at the preselected area. Because the supporter membrane is not completely flat on the glass slide, not all areas of the section are in focus (objective magnification 20x); C: section after removal of the dissected area with LPC (objective magnification 20x); D and E: isolated tumour foci in the cap of a reaction tube (objective magnification 10x).

macromolecules (Goldsworthy et al., 1999). Although the morphology of frozen sections is not optimal, snap-frozen tissue, cryostat-sectioned and mounted onto glass slides, is suitable for laser-microdissection techniques (Serth et al., 2000). Unfortunately, the surgical pathology archives of most institutions contain paraffin-embedded tissue that has been fixed in neutral buffered formalin. Formalin fixation causes widespread cross-linkage between nucleic acids and protein, which often interferes with polymerase chain reaction (PCR) amplification. The DNA extracted from such tissue is extensively fragmented, varying in size from 100–200 bp, and usually limits the size of the PCR-derived amplification. For DNA analysis 50–100 sectioned cells are required per PCR reaction.

The microdissection procedure for RNA isolation is more intensive compared to that for DNA since a large number of cells are required. Most of the recent work on RNA analysis from microdissected tissue has been performed on frozen sections. RT-PCR for the detection of single analytes has been successfully performed on single cells extracted by laser-assisted microdissection (Schutze and Lahr, 1998). However, for *in vivo* analysis of gene expression using microarrays, the limited quantity and quality of RNA isolates using laser-assisted microdissection continues to be a technical obstacle.