Electronic Supplementary Material (ESI) for Analyst

SUPPLEMENTARY INFORMATION

Infrared imaging in breast cancer: automated tissue component recognition and spectral characterization of breast cancer cells as well as tumor microenvironment

(Benard et al)

Supplementary Information is divided in two parts :

1/ Supplementary Materials & Methods

2/ Supplementary Figures & Tables

Supplementary Materials & Methods

Cell culture and processing: Six breast cancer cell lines (LY2; SKBR3; T47D; MCF-7; MDA-MB-231; MDA-MB-361) obtained from the American Type Culture Collection (ATCC, Manassas, VA) were cultivated, harvested and prepared in the Breast Cancer Translational Research Laboratory (BCTL). Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2. LY2, SKBR3, MCF-7 and MDA-MB-231 were kept in exponential growth in DMEM medium (+ L-glutamine + 4.5 g/l glucose) while T47D and MDA-MB-361 were cultivated in RPMI medium (+ L-glutamine + 25mM Hepes). The culture medium was supplemented by 10% of foetal bovine serum (FBS) and 1 % of penicillin/streptomycin (an antibiotic/antimycotic solution). Medium and penicillin/streptomycin solution were purchased from Lonza Group Ltd, Basel, Switzerland. When 80% of confluence in the culture flask was reached, cells were washed once with phosphate buffered saline (PBS) solution (Lonza Group Ltd, Basel, Switzerland). Thereafter, cells were detached by means of a five-minute treatment with 2 ml of Triple Select (Gibco, Invitrogen, Merelbeke, Belgium). The reaction was stopped by adding medium in the culture flask. The cells were pelleted by a 5-minute centrifugation at 1500 rpm. After removal of the supernatant, cells were fixed using 1 ml of formaldehyde (QPath, Labonord SAS, Templemars, France) and kept at room temperature for 2 or 3 hours. A 30-second vortex treatment was followed by a 10-minute centrifugation at 1000 rpm to remove the supernatant. PBS with 2% of agar was used in order to recover the cell pellet which was next, paraffin-embedded. A 3 µm thick section of the paraffin block was then mounted on a barium fluoride (BaF_2) window for IR imaging analysis. As well as for tissue samples, the cell pellet section was subsequently deparaffinized, rehydrated and dried.

Breast spectral database

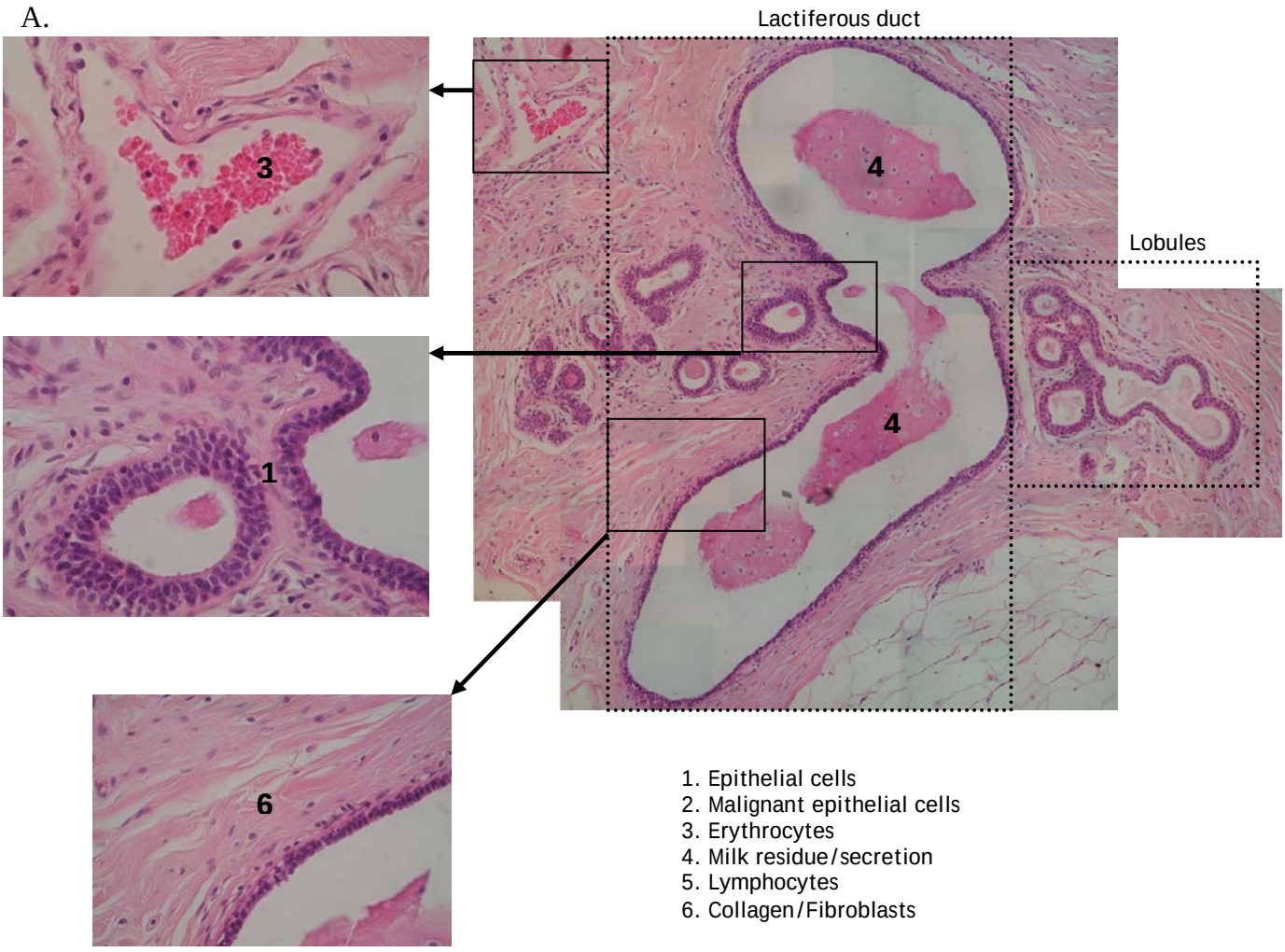
An overview of the database composition is given in Supplementary Table 1. The built-up database is large enough so that intra- and inter-class variances are adequately represented. A 2 PC clean up process was applied in order to eliminate spectra with highly different behaviour from average. For each tissue component class, a PCA analysis was computed on the 1800-1000 cm^{-1} region. The average projection on PC_1 and PC_2 and the associated standard deviation were calculated. Considering a normal distribution of the data, spectra for which the projection on PC_1 and PC_2 components beyond the standard deviation were rejected.

Supplementary figures & tables

Figure S1: Typical example of histopathological features used to build the breast spectral database. Photomicrographs of a 3 µm thick H&E stained section of an invasive grade I ductal adenocarcinoma. (A) Healthy histological structures. (B) Lymphocytic infiltration confirming immune response to the malignant proliferation. The histological types are indicated: healthy (1) and malignant (2) epithelial cells; erythrocytes (3); milk residue or secretion (4); lymphocytes (5) and extracellular matrix components (6).

Table S1 : Overview of the database composition

Figure S1



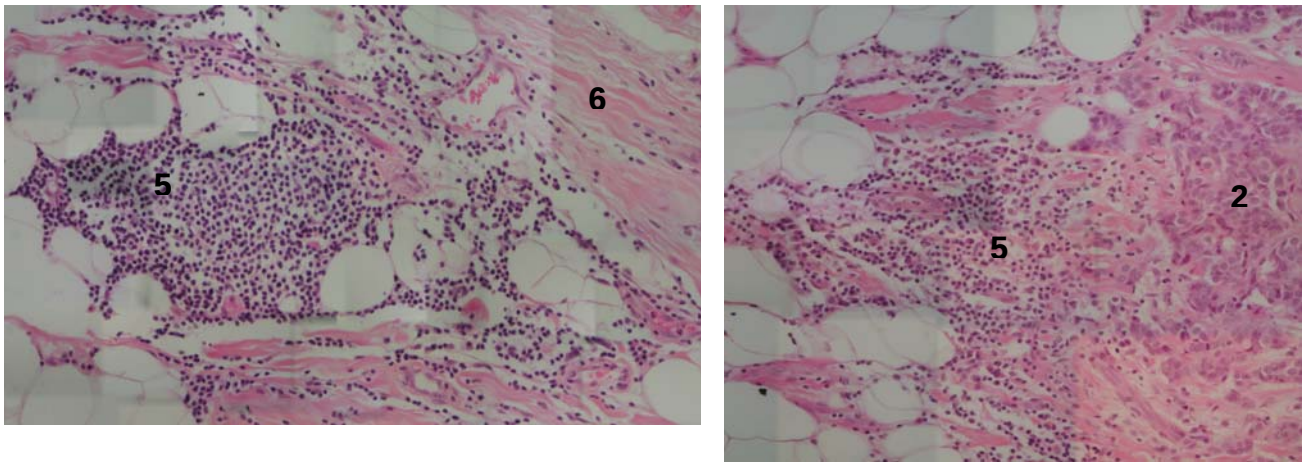


Table S1

Class name	*# database spectra*	*# spectra after 2 PC clean up*
Epithelial cells	3006	2660
Erythrocytes	1114	1023
Lymphocytes	1888	1741
Collagen & Fibroblasts	7267	6498
Protein component	293	257
Complete database	**13568**	**12179**