Electronic Supporting Information

Empirical study of the effects of acquisition parameters on FTIR hyperspectral imaging of brain tissue

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ESI 1-Digital removal of paraffin from FTIR image sections of hamster brain

Note: For the functions and data please contact david.perez-guaita@uv.es

Table of Contents

- 1. Load Data
- 2. Do Cluster without Substraction
- 3. Calculation of the relative amount of paraffin on each pixel
- 4. A spectrum of paraffin is extracted
- 5. Calculation of the contribution of paraffin
- 6. Substraction

An evaluation of the effect of the paraffin was performed. For that, it was studied the effect of paraffin following this procedure:

1. Load Data

[Im]=gCyt2G('DATA');

2. Do Cluster without Substraction

```
prep=[preprocess('default','SNV');preprocess('default','derivative')];
Imc= gCut(Im,[1100 1800] );
rng('default');
[HCA_Map1,HCA_Mask2]=gKHCAtb(Imc,[1100 1800],prep,4);
```





3. Calculation of the relative amount of paraffin on each pixel

Using the integration band of the 2850 band (2875-2833, with baseline points in the regions 2981-3036 and 2776-2808)

```
[ 0_map ] = gImgIntegrator( Im,1,2821,2875,2776,2808,2981,3036 );
```

```
figure
imagesc(0_map.data)
title ('Integrated Area (2850 cm^-^1 band)')
caxis([0 10]);
```

Integrated Area (2850 cm⁻¹ band)



4. A spectrum of paraffin is extracted

```
% Select Parafine points
paraff(:)=Im.data(127,102,:);
```

```
figure
plot(Im.yax,paraff)
xlabel('Wavenumbers cm^-^1)')
ylabel('Absorance cm^-^1)')
title ('Parraffin Spectrum')
xlim([1206 3230])
ylim([0.000 0.901])
```



5. Calculation of the contribution of paraffin

This was performed by multiplying the reference spectrum of paraffin (Step4) to the relative amount of paraffin (Step 3) of each pixel divided by the integration value of the reference spectrum of paraffin.

```
int=reshape (0_map.data,128*128,1);
```

```
Im_sub=int/0_map.data(128,103)*paraff;
```

6. Subtraction

To each pixel, it was subtracted its corresponding contribution of the paraffin. Then the KMC obtained from the nonsubtracted and subtracted image were compared.

```
Im_SUB=Im;
Im_SUB.data=reshape(Im_sub,128,128,767);
Substracted_Im=Im;
Substracted_Im.data=Im.data-Im_SUB.data;
% Substract Paraffine
Substracted_Im.datac= gCut(Substracted_Im,[1100 1800] );
rng('default')
[HCA_Map1,HCA_Mask2]=gKHCAtb(Substracted_Im.datac,[1100 1800],prep,4);
```





7. Comparison

NO SUBSTRACTION

SUBSTRACTION



Only minor dissimilarities are observed between the extracted cluster spectra. The main difference is the absorbance of the 1470 cm⁻¹ band, which decreases when the contribution of paraffin is subtracted. Results indicate that the contribution does not significantly affect the tissue characterization.