Overview

There is no quantitative results to help analyzing the exposure of a drug in regions of interest (ROI). This poster shows target exposure scoring as a tool to better understand the action of a drug. With the new module **Target Exposure** of Multimaging, an ImaBiotech software, it is now possible to **segment samples semi-automatically** in order to **obtain scores** which will help the user to determine if the drug is in **the right location,** at which concentration and compare it with other regions from the same tissue.

Target Exposure Scoring With Mass Spectrometry Imaging

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MULTIMAGING
REVEALING TISSUE SECRETS

Introduction

The evaluation of drug concentration and exposure to tissue structures, substructures or cells is a **key step** for setting up a new drug and to **anticipate** a potential lack of efficacy in tissues. These evaluations are feasible with Mass Spectrometry Imaging (MSI) but remain difficult for target exposure at high spatial resolution with high histological heterogeneity. In this study, **a new workflow** was set up using **software development** to score the absolute concentration of a drug in particular structures of tumor tissues. The objective of the workflow was to quantify a histological **drug exposure level** on the basis of the expression of its target, an enzyme, using an automated tissue annotation and quantification approaches.

Methods

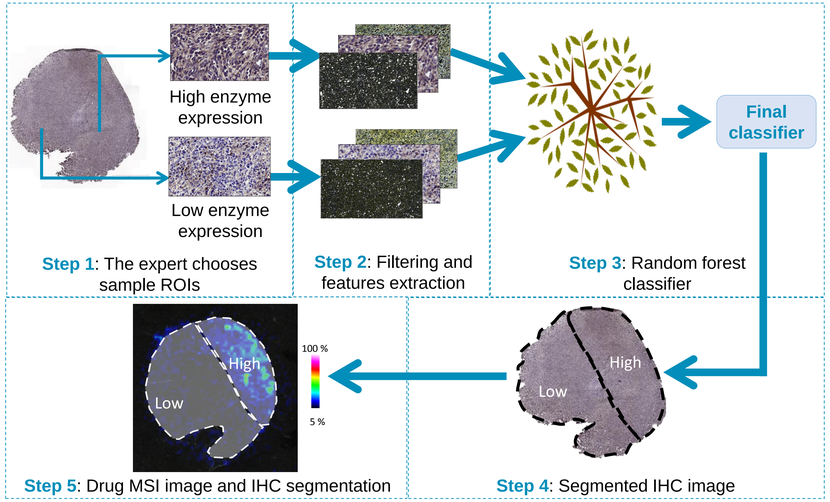
Image segmentation and **scoring** are obtained through the Target Exposure module of the Multimaging software, developed with the C# programming language.

- With IHC:
  - In order to establish the target exposure scores of the drug (that was administrated at 100 mg/kg 2h prior to mice sacrifice) for a mouse tumor models, **immunohistochemistry** (IHC) staining was performed to **highlight the enzyme** histological localization in serial tissue sections.
  - To segment the **IHC image**, the user has to select regions which represent **high and low enzyme expression levels**. Feature extraction is performed on those samples using Gaussian blur for the voxel intensity averaging, Hessian for the orientation, membrane projections for extended object detection, Sobel filter for edge detection, difference of Gaussians for measuring the size of the objects. Then a **random forest** with 200 trees, unlimited depth and 2 features is applied and the image is segmented based on the classifier.
- With MSI:
  - An image segmentation is also available based on **biomarkers** detected by MSI.
  - The software finds the area where the signal as determined by a threshold is most represented and produces a segmentation.

With the ROIs obtained and the data of the MSI, the regions' **scores** allows the determination of drug presence within a ROI and to determine specificity.

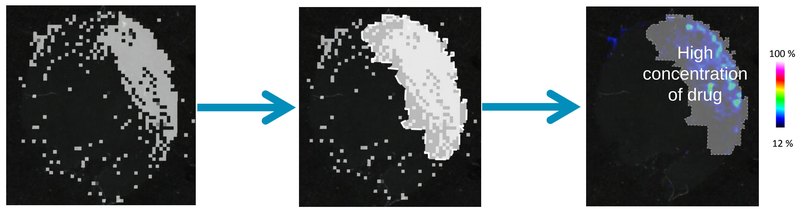
Results

Segmentation process based on IHC



Segmentation process based on MSI

The proposed segmentation is threshold dependent. For illustration purposes, the segmentation is done based on the clustered pixels representing the highest drug concentrations.



Threshold at 12% | Automated segmentation | Overlay of the segmentation on MSI

Scores calculation

The scores were calculated using the Multimaging software:

- **Quantity in entire section**: the proportion inside total sample of drug concentration for each ROI.
- **Mean of the signal or concentration to total ratio**
- **Coverage** of the drug inside the ROIs
- **Homogeneity**: If the score is between 0 and 1, the signal is homogeneous inside the ROIs
- **Mean of the signal in the sub-structures to total ratio**: doesn't take into account the positions with no signal

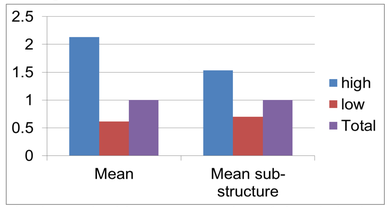
Here are the results obtained with the segmentation based on IHC with the low threshold set at 5%:

Concentrations per region type

High	Low	Total
68 µg/g	25 µg/g	42 µg/g

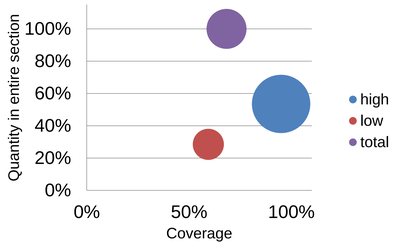
Mean ratio

The mean ratio is calculated by dividing the mean of the signals including null values of each ROIs by the mean of the signal in the total sample.



The graph clearly shows the biggest proportion of drug is in the region where enzyme staining is high. The comparison shows a big difference between the mean and the mean sub-structures. The study of the homogeneity should reveal more.

Exposure graph



The size of the bubbles corresponds to the concentrations of the regions.

The exposure graph shows that **most of the drug is inside the ROI "High".**

Homogeneity results

High	Low	Total
6.25	5.95	10.92

The homogeneity results can be interpreted as the ROIs are **heterogeneous**. It might be interesting then to study the sub-structures in which the drug would potentially be homogeneous.

Conclusions

These results confirmed that more than just reaching the organ of interest (tumor), the drug also reached its specific target. **Drug exposure specificity** was therefore confirmed thanks to the Multimaging software. As exposure at the site of action and to its specific target were identified as the most important factors for success in drug discovery and the design of chemical probes, these results showed and confirmed **the high contribution of MSI** for drug exposure and specificity to targets studies.

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