Annotation Studio

A WEB PORTAL TO SUPPORT THE DEVELOPMENT OF IMS-BASED DIAGNOSTIC ASSAYS

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Intro

Imaging Mass Spectrometry (IMS) is a promising tool to enrich pathological assessment of tissue sections with mass spectrometry-based biochemical information. With improved standardisation, quality control and method development, this integration with the clinical decision process is becoming a reality.

A central hub to manage information and coordinate efforts from labs across the globe is an important enabler towards linking pathologist expertise, IMS experiments and data analysis into a streamlined workflow.

Here we present Annotation Studio, a web-based portal that enables the direct integration of expert pathologist microscopy annotations into the IMS analysis workflow, making labeled data available for subsequent analysis and classification. Specifically, we focus on the development of a melanoma prediction model from spatially resolved protein expression profiles generated by IMS.

Sample collection

Deidentified formalin-fixed, paraffin-embedded skin biopsies from patients with suspected melanoma were provided by collaborating academic institutions and private practices. Serial sections were collected with one section used for H&E staining and a neighboring section used for IMS analysis. H&E stained sections were scanned at 20x magnification using an SCN-400 digital slide scanner (Leica Biosystems, Buffalo Grove, IL, USA) or a Huron TissueScope LE120 (Huron Digital Pathologies, St. Jacobs, Ontario, Canada), and resulting whole-slide images were imported into Annotation Studio.

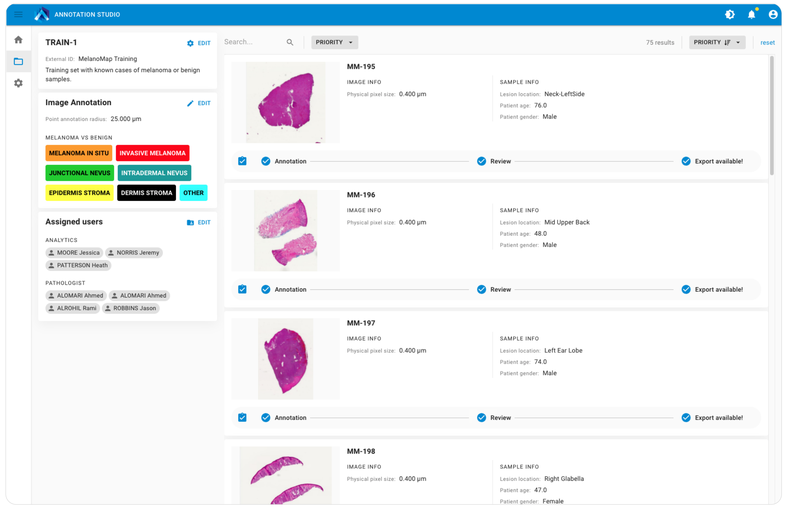


Fig 1: The project overview page provides information on a study, making it easy to search for information, and rapidly gain insight into the status of each image. Team members can easily identify actionable items via smart filtering.

Project Management

Microscopy images are uploaded from Sharepoint to Annotation Studio, where they are automatically organized into studies defined by the study manager. Each study comprises a number of labels that can be used to annotate Regions of Interest (ROIs). In this use case, the following labels are used: melanoma *in situ*, invasive melanoma, junctional nevus, intradermal nevus, uninvolved epidermis, and dermal stroma.

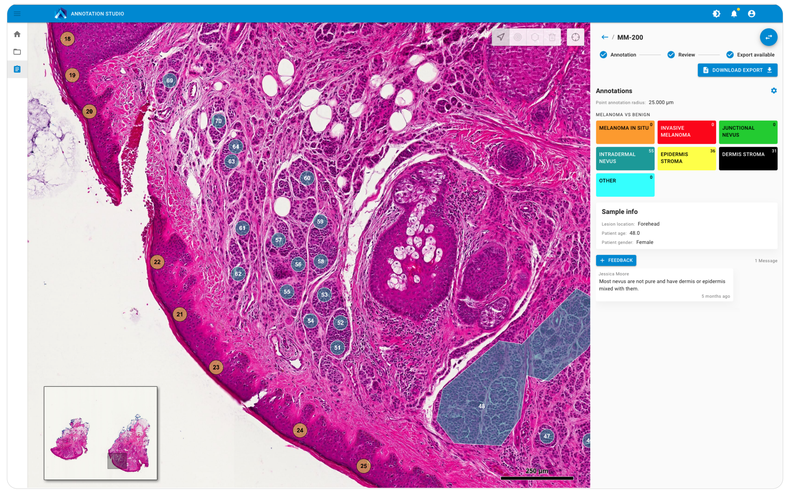
Furthermore, Annotation Studio allows the study manager to define parameters and workflows per project. In this study, the workflow encompasses the following steps:

1. Annotation by expert pathologist
2. Review by study coordinator
3. Export annotations for downstream analysis

Per project, user groups can be managed and assigned tasks through a role-based permission system, and be kept up to date of available tasks. Finally, given the diagnostics application, this role-based permission system combined with the structured workflows enables tracing actions of all users, thus facilitating high-throughput applications.

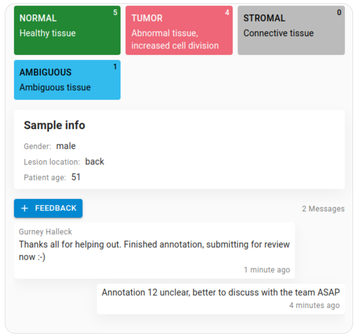
Annotation studio

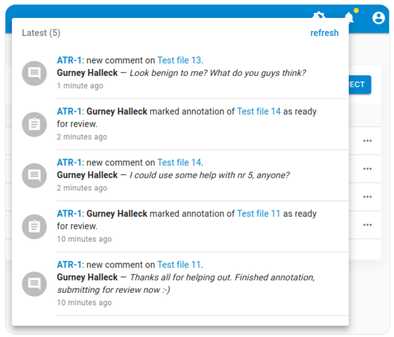
Annotation Studio features a cloud-based interactive viewer for exploration and annotation of images at full gigapixel microscopy resolution (supporting 40X zoom microscopy images). Using this viewer, pathologists mark ROIs for each sample using circles and polygons, with the labels defined at study setup. For each sample, meta-information on the sample (lesion location, patient age and gender) is displayed to assist pathologist assessment of the tissue.



Streamlined collaboration

In order to facilitate communication in large collaborative projects such as this one, Annotation Studio provides multiple options for users to communicate with each other. Users can comment on individual images, provide information on annotations, or flag potential issues, as shown below on the left. Moreover, users receive automated, personalized notifications based on their assigned roles, as shown on the right. This keeps all the information in one central location and facilitates full traceability of a study.





More info: www.aspect-analytics.com/platform/microscopy

IMS analysis

Microscopy coordinates are mapped onto a serial 6 µm thick section used for IMS analysis. The unstained section is subjected to antigen retrieval, followed by *in situ* tryptic digestion to liberate peptides from the tissue. CHCA matrix is then applied to the tissue. Matrix and trypsin are both applied using a HTX TM Sprayer. Spectra are collected from the ROIs using a Bruker ultrafleXtreme TOF-MS and tied to the labels provided by Annotation Studio (nevi vs. melanoma). Spectral data were removed from the analysis if they were obtained from damaged tissue areas or did not meet minimum mass spectral quality standards. Next, the labeled spectra can then be used to construct a spectral classification model.

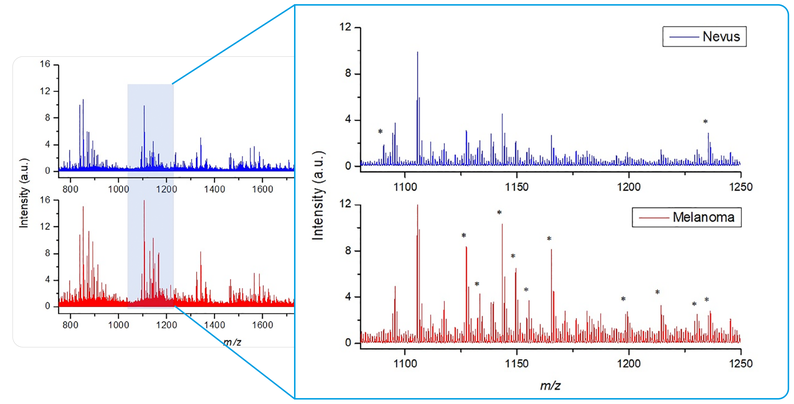


Fig. 3: Average spectrum of spots collected in a nevus sample compared to those in a melanoma sample. Asterisks indicate peaks that were selected by the model as differential between the two cases. Figure adapted from R. N. Al-Rohil, et al. [1].

Classification model

Using this annotation-based workflow we developed and validated an IMS based assay to distinguish between melanoma and nevi as described in R. N. Al-Rohil, et al. [1]. The study included a total of 333 unambiguous melanocytic neoplasms, divided into a training (n = 241) and test set (n = 92).

Based on the collected IMS data collected, an **ensemble support vector machine (SVM) classifier** was trained based on all spectra in the training set, yielding a 97.3% sensitivity and 97.5% specificity on the spectra of the independent test set. Clustered cross validation was used to prevent information leakage from spectra collected from the same tissue sample, thus preventing overfitting of the model.

As multiple spectra are collected per sample, classifications per spectrum need to be pooled at the sample level, in order to determine the likelihood that a sample is a melanoma. We applied quality metrics to ensure that only high-confidence spectra were included for the classification. Only spectra with a prediction probability greater than or equal to 95% are included in the sample-level scoring.

The **sample-level scoring algorithm** computes the simple ratio of spots that predict melanoma relative to that sample's total number of spots, giving a scale from 0 to 1. Samples with a score ≥0.85 are classified as "melanoma," samples with a score ≤0.15 as "benign," and samples with scores >0.15 and <0.85 as "indeterminate." The sample-level scoring algorithm of the test set classified 39 of 40 benign nevi samples for a **specificity of 97.5%,** 36 of 37 melanoma samples for a **sensitivity of 97.3%**, and 15 samples as indeterminate (n = 9 benign nevi and n = 6 melanoma).

Conclusion

Annotation Study provides a valuable framework to **coordinate and streamline high-throughput studies** where pathologist expertise, IMS experiments and data analysis are combined. This workflow enabled the construction and high-throughput use of an **IMS-based classification model**, showing **very good sensitivity and specificity** in distinguishing benign nevi samples from melanoma samples.

References

[1] R.N. Al-Rohil, J.L. Moore, N.H. Patterson, S. Nicholson, N. Verbeeck, M. Claesen, J.Z. Muhammad, R.M. Caprioli, J.L. Norris, S. Kantrow, M. Compton, J. Robbins, A.K. Alomari; ***Diagnosis of Melanoma by Imaging Mass Spectrometry: Development and Validation of a Melanoma Prediction Model,*** *Journal of cutaneous pathology, 2021*