



Metbots: Metabolomics Robots for Precision Viticulture

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Abstract. Metabolomics is paramount for precision agriculture. Knowing the metabolic state of the vine and its implication for grape quality is of outermost importance for viticulture and wine industry. The MetBots system is a metabolomics precision agriculture platform, for automated monitoring of vineyards, providing geo-referenced metabolic images that are correlated and interpreted by an artificial intelligence self-learning system for aiding precise viticultural practices. Results can further be used to analyze the plant metabolic response by genome-scale models. In this research, we introduce the system main components: (i) robotic platform; (ii) autonomous navigation; (iii) sampling arm manipulation; (iv) spectroscopy systems; and (v) non-invasive, real-time metabolic hyper-spectral imaging monitoring of vineyards. The full potential of the Metbots system is revealed when metabolic data and images are analyzed by big data AI and systems biology vine plant models, establishing a new age of molecular biology precision agriculture.

Keywords: Metabolism · Spectroscopy · Artificial intelligence · Autonomous systems · Non-invasive · ‘In-vivo’ monitoring

1 Introduction

Wine is a highly complex biotechnology product. It all begins at the vineyard, where the interaction of soil, climate and plant physiology, determines the desired characteristics. Producing high-quality wines on a constant basis is the major goal of precision viticulture.

Multi-spectral satellite, drone imaging and ‘in-situ’ sensors, when complemented with pattern recognition and artificial intelligence, are today the state-of-the-art of the 21st century viticulture [34]. Although aerial technologies are able to cover significant land masses [17, 33, 37], almost no information about the plant metabolism, grape quality and soil nutrients, is possible to be obtained from these methods. A characteristic example is the normalized difference vegetation index (NDVI). NDVI is poorly correlated to important metabolites, such

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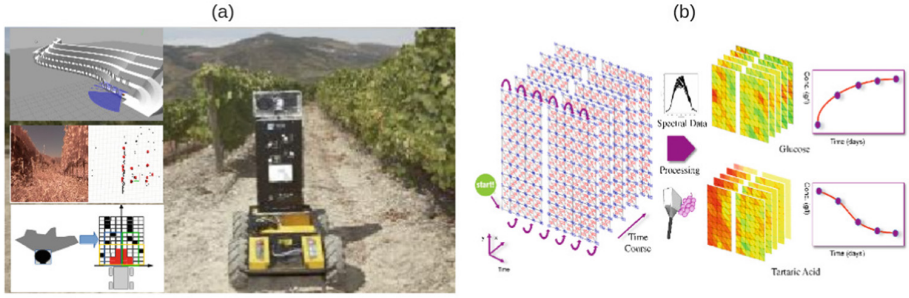


Fig. 1. Metbots metabolic imaging: (a) AgRob V16 robotic platform (navigation, positioning, sampling); (b) geo-referenced uv-vis-swnir hyperspectral metabolic imaging

as, the phenolic composition [17]. Only ‘in-situ’ technology is able to provide rich metabolic information.

‘In-situ’ technology is available to viticulture, such as: (i) computer vision: determining production yield [1,6]; (ii) soil composition (vis-swnir, x-ray fluorescence, LIBS) [31,32], and (iii) grape composition [23,36]. Uv-vis-swnir spectroscopy has shown to be a robust metabolomics tool in viticulture [8,23]. Parameters such as: degree Brix, total soluble solids [5,18], total acidity and reducing sugars and acids [9], and polyphenols [15]. Furthermore, results from ‘in-situ’ systems [12,36] have shown random sampling of grapes during traditional maturation control, and cannot describe the ‘terroir’ nor viticultural practices impact on grape quality. High-resolution geo-referenced metabolic imaging technology is able to characterize the impact of soil, climate and viticultural practices on grape quality, with emphasis on sugar/acids, anthocyanin, beta-carotene and lutein [23].

We developed a precision geo-referenced metabolic imaging using uv-vis-swnir spectroscopy [24]. The system accuracy was significantly increased by developing a big data self-learning AI methodology, for the accurate quantification and classification of spectral information, under complex variability and multi-scale interference. This new method has allowed to decrease most of quantification errors of previous technologies to low quantification errors [20].

Grape maturation was followed from May to September in experimental fields, using a geo-referenced sampling mesh (spectra and grape samples were collected at nodal points), mostly in Douro, Dão and Ribatejo, to grape varieties such as Tinta Roriz, Touriga Franca, Syrah, Touriga Nacional and Pinot Noir. The developed system performs geo-referenced metabolic images to: glucose, fructose, tartaric and malic acids, neoxanthins, zeoxanthins, anthocyanins, beta-carotene and lutein [13,21–23]. The user can visualize the metabolic evolution of grape maturation with a viewer software, and navigate along the field for obtaining the grape composition at the points of sampling an in-between, by the finite element method.

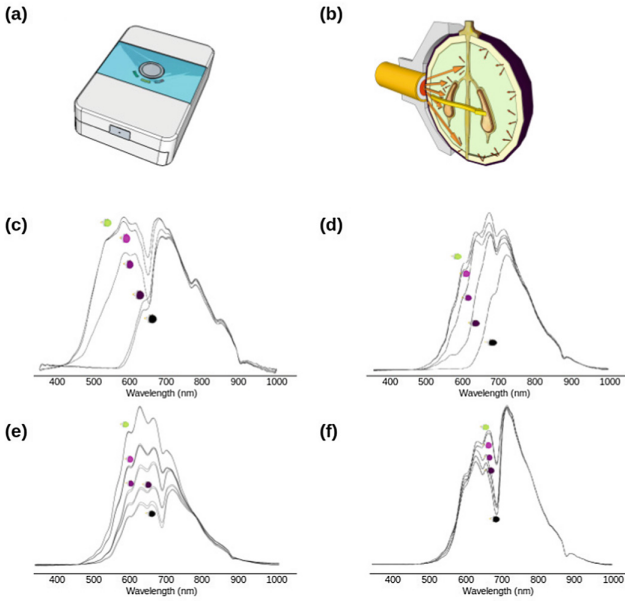


Fig. 2. Metbots IoT Spectroscopy System: (a) IoT Spectroscopy device; (b) Grape structures captured by light; and spectra along maturation from (c) grapes; (d) grape skins; (e) grape pulps and (f) grape seeds. The capacity of measuring these three structures is of most importance to wine quality, as different compounds are present in skin, pulp and seeds.

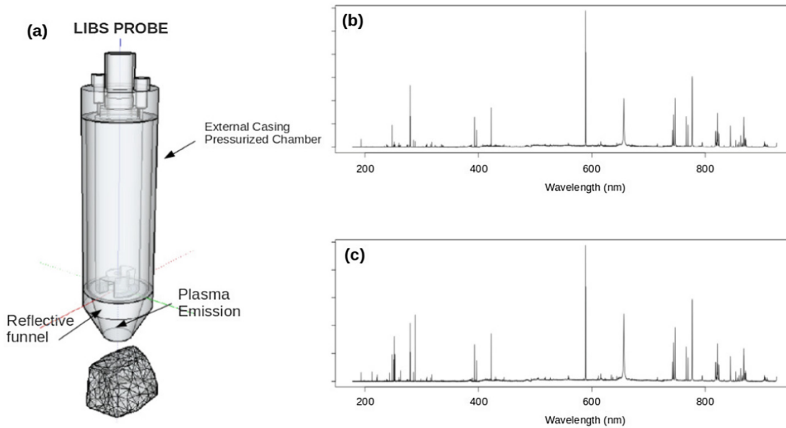


Fig. 3. Metbots LIBS system: (a) LIBS probe for agricultural applications; (b) leaf spectra without pesticide treatment; (c) leaf spectra with pesticide treatment.

Uv-vis-swnir is capable to show that: (i) the soil composition influences directly the grape maturation process; and (ii) irrigation regime influence on technical maturation (sugars/acids), anthocyanins and beta-carotenes [13,23].

The system is capable of monitoring chlorophyll-like compounds and carotenoids present in the grapes. These are important precursors of norisoprenoids, a key constituent of high-quality Port wine aromas. During Port wine ageing due to oxidation, beta-carotenes levels decrease, giving rise to TDN, vitispirane and TCH. In lutein supplemented wines, b-damascenone increases, and when supplemented with b-carotene, b-ionone and b-cyclocitral levels increase 2.5 times [13]. Carotenoids are known to depend on cultivar, climate conditions, viticultural region, irrigation, sunlight exposure and ripening stage [29].

Carotenoids are mostly present in the grape skin (65% of total berry carotenoids: lutein, xanthophyll and b-carotene), being easily detected by spectroscopy (Fig. 2). Carotenes are considered as light harvesters, as a protection to excessive light in unripe grapes. However, during maturation, grapes exposed to light have lower levels of carotene [8]. Oliveira et al. [29] also found that terrain elevation (lower temperatures and higher humidity) lead to the production of higher contents of carotenoids. Also, lower vegetative indexes correspond to lower carotenoid concentrations. Furthermore, soils with low water retention capacity always produce grapes with higher levels of carotenoids [29].

Figure 2 presents the current system under design and how it measures grape composition. It can work as portable miniature analyzer IoT device, that connects to a mobile phone or the AgRob V16 robot. The reflection probe in Fig. 2b uses a special designed fibre optics reflection probe, to provide spectral integration of the grape internal anatomy. With this configuration, the optics can obtain spectral information about the skin, pulp and seeds. Spectral integration is shown in Fig. 2c–f, where the grape spectra Fig. 2c is the integration of the skin (Fig. 2d), pulp (Fig. 2e) and seeds (Fig. 2e) spectra.

The miniaturized spectrometer can be used by a human to record the spectra (Fig. 2). It is not adequate for covering vast areas and difficult terrains, such as the Douro valley, and therefore this task is automated in Metbots. There is still very few available robots for agricultural applications. In fact, in the last two decades some robotic solutions were developed for specific tasks in agriculture, however, due to the characteristics of the terrain and the type of crops used, many of these solutions are not easily scalable and/or reproducible to other farms. The INESC TEC robotics lab has been developing steep slope robotic platforms [35] (AgRob V16 - agrob.inesctec.pt) and ROMOVI (P2020 project) [26] for operating in the Douro valley terrains, that overcame: GPS signal problems, harsh terrain conditions that limit instrumentation and slopes impose precise path planning.

The present generation of robots uses our developed VineSLAM system [35] takes into consideration the natural and artificial features of the vineyard to recognize the localization, compensating for poor GPS accuracy. Tests of AgRob V16 in a real steep slope vineyard show that this platform can overcome ditches,



Fig. 4. Manipulation state machine

rocks and high slopes (30%). With a robust localization system, it can perform autonomously a crop monitoring task (crop yield, soil/air temperature/humidity and crop water stress index), being cost effective for the end-user.

The MetBots project main objective is to research and develop a robotic and AI system for metabolomics precision agriculture, using uv-vis-swnir and laser induced breakdown spectroscopy (LIBS), in conjunction with the AgRob V16 system (Fig. 1), to monitor the plant metabolism.

The project is divided into three main parts: (i) robotics and sensors - spectroscopy sensors are incorporated with the robotic platform for automatic monitoring; (ii) system infrastructure - where all the information is stored and processed by self-learning AI technology; and (iii) field tests - to validate the efficiency of automatic monitoring and diagnosis in real scenarios.

The research project is developed by Institute for Systems and Computer Engineering, Technology and Science (INESC TEC) and Duriense Viticultural Development Association Laboratories (ADVID), aiming to implement metabolic diagnosis in precision viticulture at the Portuguese Douro Valley wine region.

2 Manipulation

For a fully intelligent and autonomous system, it is required a robotic arm capable of handling the spectroscopy sensor, in order to copy the human behaviour on this task. However, autonomous sensing problem cannot be solved as some industrial problems, where static trajectories are predefined and the robot executes them repetitively. Instead, this case needs to accomplish active perception solutions [2] for grape recognition. So a complete manipulation solution has to accomplish the following steps: bunch of grapes and grapes detection and recognition [3, 25], path planning [14, 27], and trajectory control.

The Fig. 4 states the different stages of the manipulation sensing process. On rest state, a manipulator included sensor will continuously look to the vineyard searching for grapes bunches [3]. When a new bunch is detected, the system chooses if this bunch will be sampled. In a positive case, a gross planning is done to a near point of the bunch [14] and after a final path planning is made relative to the end-effector frame until the selected grape [25, 27]. Finally, the manipulator returns to an initial standard position through a global path plan.

Table 1. Average quantification benchmark results for Tinta Roriz, Touriga Franca, Syrah and Cardinal cultivars

Parameter	Range	DL	R ²
Degree Brix	5.0–25.0	5.2	0.78
Glucose	1.3–160.0	7.3	0.77
Fructose	3.3–100.0	6.3	0.78
Malic Acid	1.0–10.0	0.37	0.82
Tartaric Acid	1.0–8.0	0.30	0.76

3 Spectroscopy Measurement Control

High-quality spectra, as shown in Fig. 2c, are necessary for accurate metabolite quantification. The spectral probe must contact the grape skin in order to avoid any reflections into the pin-hole receiver fiber (Fig. 2b). External fibers illuminate all regions of the grape, in order to obtain an integration of all grape structures (skin, pulp and seeds) (Fig. 2d to f).

Once the robotic arm positions the probe at 0.5 cm of the grape to be analyzed, the spectrometer data assumes positioning control. The spectra pattern is used to know if the probe is in the correct position, by the following procedure: (i) record spectra with the maximum power of the light source, while pushing forward the probe, until no surface reflection from the grape is detected; (ii) adjust the light source power and integration time for optimal spectra recording inside the linear region of quantification; and (iii) record the grape spectra.

The spectra pattern is analyzed by the projection into a principal components feature space, where a linear discriminant model, discriminates between the reflected light spectra and grape spectra.

4 Spectroscopy Processing

Vis-swnir spectrum were pre-processed to remove artifacts, such as, effects of baseline shifts, Mie and Rayleigh scattering and stray-light [7, 10].

Correlation between spectra and grape composition was modelled by partial least squares regression (PLS) [11]. PLS is a linear multivariate model based on latent variables (eigenvectors/eigenvalues) that maximizes the co-variance matrix ($\mathbf{X}^t\mathbf{Y}$) between the spectrum matrix (\mathbf{X}) and the analytical chemistry data (\mathbf{Y}): $\mathbf{Y} = \mathbf{X}b + e$; where b translates the linear combination that projects the spectral information into the analytical chemistry data [30] (Fig. 5).

Grape variety may influence how we can relate the composition and spectral variation. Therefore, independent calibration predictive models were built for the different grape varieties: Touriga Nacional, Touriga Franca, Tinta Roriz, Syrah; and further table grapes of Cardinal variety. Representative samples across the different composition levels are paramount to build a globally stable PLS model.

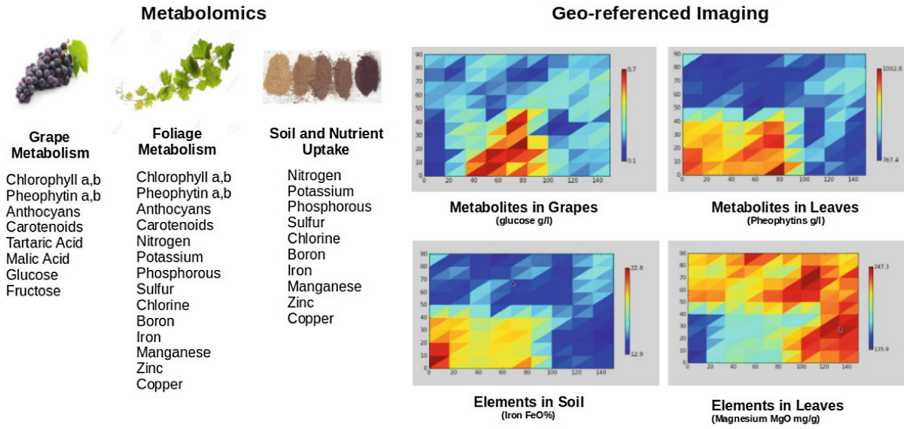


Fig. 5. Metbots metabolic geo-referenced uv-vis-swnir imaging in grapes, foliage and soil composition.

Therefore, each level of sugars and acids has the same level of representation in the global PLS model [4].

The PLS model describes a linear relationship between composition and spectral features. Such means that the correct number of latent variables (LV) is considered optimal, once it balances bias vs variance. In PLS modelling, the optimal number of LVs is considered the global minimum of the test set. This is the case in simple systems, where prediction errors increase due uncorrelated information present in the spectra. This does not happen when correlating grape spectra shown in Fig. 2 with composition, as the spectra is extremely rich in information about the grape composition. In this case, the PRESS continuously decreases, and no saddle point exits. Such means that we must ensure a new way of choosing the correct number of LVs that ensure model linearity, at the expense of higher variance, so that the PLS model can be used as a generalized linear model, capable of quantification along the range of variation.

To mitigate bias-variance and PLS linearity, we devised the following two step validation scheme:

- i. global cross-validation: we used all data to develop the global model cross-validation PRESS curve and select solely the number of LVs that are with the steepest descent on PRESS optimization to the second step. The procedure is as follows: we devised the composition range into 50 intervals with $n-k$ samples at each level. The remaining k samples were used to perform the model validation at each level. The cross-validation PRESS is computed for the k validation datasets, until all data is used to build validation datasets. The Kennard and Stone algorithm [16] was used to select representative samples at each level, so that all natural variability is all accounted for in the regression model, minimizing the risk of biased models. This scheme allows to set-up an uniform sampling bootstrapping and cross-validation [19].

- ii. extrapolation cross-validation: using only the selected number of LVs in the previous step, we now must select the minimum number of LVs that allow to maintain a stable linear model, by minimizing biased predictions under extrapolation, so that:

$$\text{Range}(\%) = \frac{\max(\mathbf{Y}) - \min(\mathbf{Y})}{\max(\mathbf{Y}^c) - \min(\mathbf{Y}^c)} \quad (1)$$

where \mathbf{Y}^c is the dataset used for the calibration, and \mathbf{Y} the corresponding global calibration dataset. The objective of the 2nd step is to obtain the minimum number of LVs that hold a maximal range of prediction with minimal range of training set, so that a globally stable linear unbiased calibration is obtained. To further understand if the extracted PLS coefficients are statistically stable, 1000 bootstrap samples with $n-1$ samples were used to determine the coefficients variation and significance assessed by the t-student test [19, 28].

This method allows to derive quantification for the major constituents of the grape, as presented in Table 1. PLS modeling allows to reasonably quantify ‘in-situ’ the degree Brix, glucose, fructose, malic and tartaric acids. The project aims to develop model calibrations also for: chlorophylls a and b, pheophytins a and b, anthocyanins and carotenoids, using uv-vis-swnir. The project will further explore the measurement of elements in soils and leaves (e.g. N, Fe, Cu, S, Cl, Mn, Zn, P, K) using LIBS spectroscopy, as well as, determine the amounts of applied agro-chemicals, as presented in Fig. 3, where trace levels of pesticide can be discriminated between control (Fig. 3b) and treated leaf (Fig. 3c).

5 Hyperspectral and Metabolic Imaging

Hyperspectral images are assembled from individual spectral measurements at nodal points from a pre-established geo-referenced mesh that minimizes sampling time (see Fig. 1). The robot is set into a pre-determined path to stop at specific vines, where it collects geo-referenced measurements. A part of this measurements is uv-vis-swnir and LIBS spectra. In uv-vis-swnir measurements, three grapes are measured at each vine. Each measurement takes approximately 1 min. Images are taken with 100 nodal points density per hectare, which can be done in approximately 2 h, depending on the ‘terroir’ topographic features. The metabolic image is reconstructed by inference of composition from spectral PLS regression models at each node. Metabolite gradients are interpolated using triangular finite elements, so that visualization is continuous across the vineyard mesh. Metabolic images can be validated by physical collection of samples at selected nodal points and performing corresponding laboratory chemical analysis.

The full potential of metabolic imaging using uv-vis-swnir and LIBS spectroscopy is presented in Fig. 4. Uv-vis-swnir and LIBS spectroscopy provides a comprehensive characterization of metabolites and nutrients, such as: chlorophylls a and b, pheophytins a and b, anthocyanins, carotenoids, tartaric and

malic acids, degree Brix, glucose and fructose; as well as, major inorganic nutrients (nitrogen, potassium, phosphorous, sulfur, iron, magnesium, manganese, boron, zinc or copper). These parameters can be, for the first time, geo-referenced and compiled for grapes, leaves and soil; providing a significantly more complete set of information about the vine metabolism than previous technologies.

Metbots records information about plant metabolism. It is a new tool that opens precision viticulture to molecular biology viticultural management practices. Molecular information will allow to use data science/ artificial intelligence, to both analyze and predict the effects of agricultural practices, as well as, to make use of state of plant genome scale models, inferring the vine plant physiological response at the genetic, proteomic and metabolome levels. The Metbots project hopes to bring to the field, precision metabolomics and molecular biology, allowing producers and researchers to confront knowledge obtained under controlled laboratory or field test conditions, against what is observed in the open field by large scale sampling and integrative data from climate-soil-plant.

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