

## 8. Non-invasive In-Ovo Sexing of Chicken Eggs using Raman Spectroscopy and Hyperspectral Imaging

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In order to meet the demand for consumption eggs, billions of specially bred layer chickens are hatched every year. Serving no purpose in the industry, over 420 million one day old male chicks are culled every year in Europe. Current, accurate (>95%) commercial in-ovo sexing techniques are unfit for sexing before day 9 of incubation (E9) and their invasive nature imposes a risk for bacterial infection. With upcoming new legislation aiming to outlaw the culling of chicken embryos after E7, there is an urgent need for the development of non-invasive early in-ovo chicken sexing methods. Reports relying on spectroscopy techniques have been abundant in recent years, yet relatively few have been demonstrated in practice. The most compelling of the explored research techniques relies on the analysis of extraembryonic blood vessels using Raman and fluorescence spectroscopies, enabling sexing with good accuracy at E4 without disrupting the egg membranes. Other researchers turned to machine vision to determine blood vessel geometry from conventional photographs to perform in-ovo sexing with good accuracy at E4. Hyperspectral imaging (HSI) has the potential for commercial deployment of in-ovo sexing, as texture analysis of HSI in the near-infrared can facilitate sexing with excellent accuracy, even before incubation. While these techniques have indisputable potential in in-ovo sexing, most of these efforts failed to meet the high standards for sexing accuracy, while others were faced insurmountable challenges during upscaling or when generalizing their approach to other breeds. This PhD project envisions the determination of spectral sex-specific digital biomarkers in the eggshell using Raman imaging complementary to visible-light HSI in enabling early and accurate non-invasive in-ovo sexing. Initial identification of novel biomarkers in white and brown eggs will be performed using Raman and elaborate chemometric analysis with sex determined using PCR as the cross-validation method. Likewise, informative spectral, morphological and textural features will distinctively be identified in HSI data obtained for the same eggs. State of the art data fusion strategies will be employed to expedite joint analysis and training of classification algorithms on Raman imaging and HSI data for in-ovo sexing. Digital biomarker model evaluated by deep learning approaches as a tool will be explored as part of this PhD project in the screening for early detection of sex in the eggs.