Electronic Supplementary Information (ESI): Diagnosis of early-stage nasopharyngeal carcinoma using ultraviolet autofluorescence excitation-emission matrix spectroscopy and parallel factor analysis

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In this Electronic Supplementary Information (ESI), the autofluorescence (AF) properties of aromatic amino acids tyrosine (Tyr) and tryptophan (Trp) are discussed, followed by a comparison of the AF spectra from pure Tyr and Trp, to normal nasopharyngeal and nasopharyngeal carcinoma (NPC) tissues. The potential contribution of Tyr in NPC is then considered in order to validate the effectiveness of the three reported fluorophores (Trp, collagen, and elastin) in early-stage diagnosis of NPC.

Trp, Tyr, and phenylalanine (Phe) are three aromatic amino acids with respective quantum yields (QYs) of 0.2, 0.14, and 0.04.[1] The low QY of Phe does not contribute significantly to the cumulative AF of the aromatic compounds. Tyr AF is also generally not detected, despite having a QY closer to Trp than Phe. Two main characteristics could explain this observation. First, the Tyr emission band is 34 nm at full width half maximum (FWHM) and has a peak emission around 300 nm, while the Trp emission band is 60 nm at FWHM and has a peak emission around 330 nm.[2] The broader emission band at longer wavelengths increases Trp AF detection. Second, energy transfer from Tyr to Trp is quite common.[1] The consequential quenching of Tyr is seen to increase the overall dominating AF of Trp.[2]

To experimentally observe the AF contribution of Tyr compared to Trp in nasopharyngeal tissue, emission spectra of pure Tyr and Trp were collected at peak excitation wavelengths from 270 nm to 300 nm.[1, 3] The excitation wavelength was incremented by 10 nm to maintain consistency with the optimized tissue data experimental protocol. The Tyr and Trp AF spectra were plotted against the corresponding AF spectra from normal and NPC tissue. These spectral bands were consistent with the PARAFAC model contour plot shown in Fig. 5a of the manuscript. The results in Fig. 1 are presented on a uniform x and y axis.

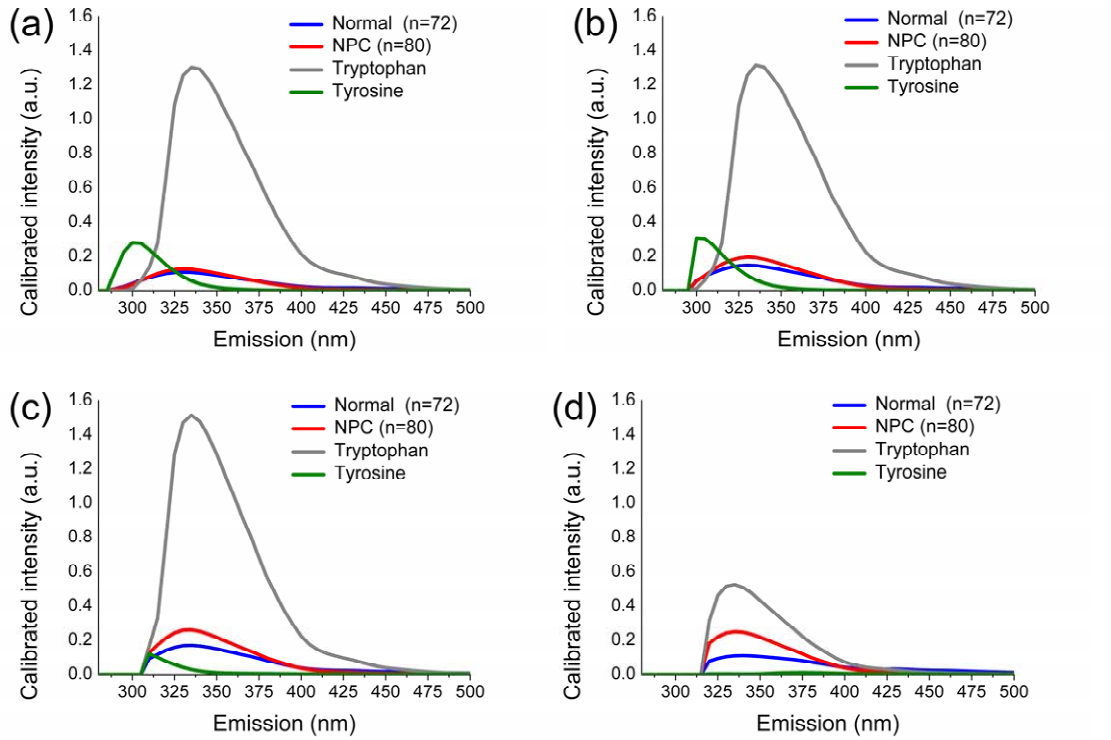

Fig. 1: Pure Tyr, Trp, normal and NPC tissue emission spectra under a) 270 nm, b) 280 nm, c) 290 nm, and d) 300 nm excitation.

Trp clearly produced the greatest AF emission between 325 nm – 335 nm under all four excitation wavelengths. This dominant signal can be seen in the line shape and peak position of both normal and NPC tissue spectra. Tyr produced an optimal AF signal between 295 nm – 305 nm under 270 nm excitation, but was predictably lower compared to Trp. (Note that the perception of a changing Tyr line shape is due to the shifting excitation-emission on the uniform x-axis.) Our results suggest that the Tyr AF contribution was unobservable based on the lack of significant spectral features in the tissue data between the peak emission band from 290 nm – 310 nm.[1, 3] As seen in Fig. 1(a-d) above, we confirmed that the minor contribution of Tyr to the nasopharyngeal tissue AF would not have been resolved in our PARAFAC model. The three factor constructed PARAFAC model retained a degree of simplicity, and thus the reported parameters enabled a consistent analysis while accurately describing each dominant tissue component. Future work may consider a model of increased complexity that would more accurately account for the Tyr contribution.

Despite the reduced AF emission compared to Trp, Tyr has also been shown to be an important endogenous fluorophore that contributes to the fluorescent properties of diseased tissue. For example, Tyr phosphorylation and alkaline phosphatase (ALP) have been molecularly implicated in various diseases, including NPC.[4-7] It is possible

that an increase in ALP activity due to carcinogenesis could lead to increased Tyr absorbance, possibly causing an increasing Trp-like effect due to their similar molecular properties. Although our preliminary results shown in the manuscript and Fig. 1 of this ESI support our experimentally observed *increase* of Trp AF signal within NPC tissue, studies have also found a *decrease* of Trp in cancer patients due to an increase of an immunosuppressive enzyme, indoleamine 2,3-dioxygenase, in tumor cells.[8] This alteration has been shown to facilitate cancer progression by affecting T-cell proliferation and tryptophan contribution,[9] a pathway that has long been recognized in cancer patients[10] and recently shown in NPC patients, resulting in extreme tryptophan depletion at tumor sites.[11] Further molecular studies are necessary to pinpoint the differences observed in these results.

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