

Volumetric one-photon UVA hyperspectral light sheet imaging in mouse pre-implantation embryos - INVITED

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Abstract. UVA one-photon hyperspectral light sheet imaging was performed here to capture and reconstruct three dimensional metabolic maps of mouse embryos during development. We explored numerically and experimentally the advantages of phasor-based hyperspectral approach for detecting embryo metabolism with a single wavelength excitation compared to the conventional detection approach using bandpass filters.

1 Introduction

Fluorescence imaging of the metabolic cofactors NADH (reduced nicotinamide adenine dinucleotide) and FAD (flavin adenine dinucleotide) has long been used to characterize the metabolic activity of a large variety of biological samples as an indicator of health. Recently, optical imaging has been gaining interest in the field of embryology with its capacity to extract metabolic information of embryos. The ability to identify embryos with the highest potential for implantation that result in successful pregnancies is a real challenge in the context of in-vitro fertilization procedures, outside of invasive biopsy or subjective morphokinetic assessment. Therefore, rapid, non-invasive, accurate and easy-to-implement imaging techniques capable of capturing 3D metabolic information on embryos prior to transfer for implantation are attractive for future clinical applications. Light sheet microscopy (LSM) fits the bill in this sense, due to its high-speed imaging capability and phototoxicity mitigation with the confinement of the excitation volume just to the detection plane. Considering these advantages, LSM has been applied to metabolic imaging using a two-photon approach [1] or a single photon with two excitation wavelengths [2]. To ensure ease of use and portability of detection tools for clinical studies, the complexity of imaging systems must be minimized. In this regard, one-photon single excitation LSM would be beneficial.

Here, we propose a one-photon single excitation LSM combined with a spectral phasor-based hyperspectral detection for fast metabolic imaging. The key challenge of single excitation is the ability to co-excite NADH and FAD while probing changes in their contributions to total fluorescence with high sensitivity. By use of a numerical study, we show how the redox ratio (RR) - defined as $RR = FAD/(FAD+NADH)$ - calculated with spectral phasor analysis compares with the

conventional detection method based on bandpass filter transmission. Further, we experimentally evaluated RR values of preimplantation mouse embryos during development from 3D metabolic imaging acquired with a one-photon UVA excitation light sheet for conventional detection versus hyperspectral phasor-based detection method.

2 Results

2.1 Numeric study

We first present a numerical comparison between the RR values evaluated from the spectral phasor analysis and the spectral filtering with bandpass filters. Analysis of the spectra is based on the Fourier transformation of the fluorescence emission spectrum in the spectral domain into two coefficients (G,S) plotted on a polar graph. A special feature of this analysis is that the coordinates of the mixed solution are located on a line between the phasor coordinates of the pure NADH and FAD solution. We show that the RR associated with a mixed solution of NADH and FAD and described by the phasor coordinates $P_{\text{mixed}} (G_{\text{mixed}}, S_{\text{mixed}})$ can be determined by $RR = \frac{\|P_{\text{mixed}} - P_{\text{NADH}}\|}{\|P_{\text{NADH}} - P_{\text{FAD}}\|}$ where P_{NADH} and P_{FAD} are the phasor coordinates of NADH and FAD respectively. From the emission spectra of mixed solutions of NADH and FAD shown in Figure 2.a, we calculated the phasor coordinates (see Figure 2.b), then derived associated RR values. We then compared these RR values computed using the phasor-based hyperspectral analysis versus conventional analysis with bandpass filters. As shown in Figure 2.c, the redox values obtained with the hyperspectral approach are more precise as they describe variations between 0 and 1 when the concentration dilution varies between 0 (pure NADH) and 100% (pure FAD). The calculated STD/gradient also shows a higher

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degree of accuracy from the hyperspectral measurements with the spectral phasor (see Figure 2.d).

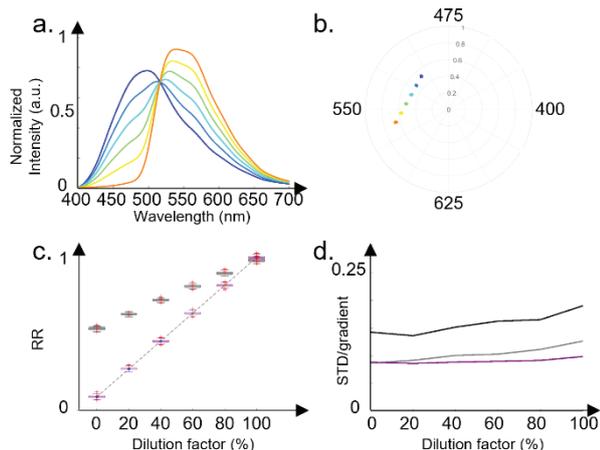


Fig. 1. a. Emission spectra of dilutions with mixed NADH (blue curve) and FAD (red curve). b. Polar plot with corresponding phasor coordinates of solutions from (a). Plots of c. RR values estimated with conventional (black: thin bandwidth, grey: large bandwidth) and hyperspectral approach (purple) and d. STD/gradient values.

2.2 Volumetric metabolic imaging in mouse embryos

We next experimentally assessed metabolic activity in mouse embryos using a custom-built phasor-based hyperspectral light sheet with a UVA excitation in the geometry illustrated in Figure 2. The phasor-based hyperspectral approach used here was proposed by Hedde et al. [1]. It consists of an in-hardware implementation of the spectral phasor analysis which relies on the use of two specific filters with transmissions for a cosine and sine profile over the visible range placed in the detection path.

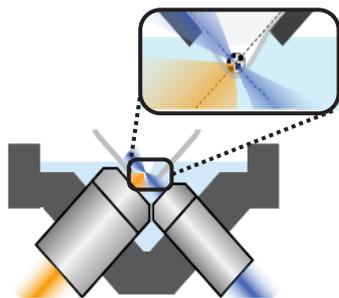


Fig. 2. Cross-sectional diagram of the imaging geometry with the excitation light sheet beam (blue) and the collection of fluorescent light (orange) emitted from the sample. The sample is placed on a V-shaped holder on top of the objectives (grey).

Three-dimensional metabolic imaging was performed on preimplantation mouse embryos at the 2-, 4-, 8-cell, morula and blastocyst stage. Figure 3 shows an example of a 3D reconstruction of metabolic activity using the phasor-based hyperspectral detection method in a blastocyst-stage embryo.

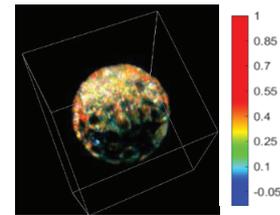


Fig. 3. Example of a volumetric reconstruction of the metabolic content of a blastocyst stage embryo.

We further investigated how the RR varies during embryo development using both the HS and the conventional approaches. As shown in Figure 4, statistical analysis reveals a significant increase from the 4-cell stage compared to later stages of development as detected with the HS method, characteristic of embryonic development [3]. Compared to the RR values obtained with bandpass filters (no changes detected in early development), the RR values obtained with HS were found to be more sensitive and accurate at detecting changes in metabolic activity during development.

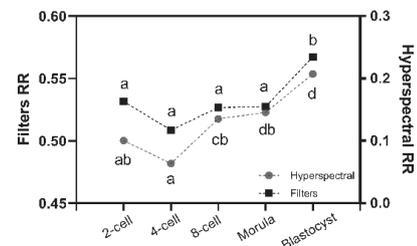


Fig. 4. Average RR values measured over the entire volume of 2-, 4-, 8-cell, morula and blastocyst-stage mouse embryos. Different alphabets indicate statistical significance ($P < 0.05$) between developmental stages.

3 Conclusion

In conclusion, we have demonstrated an original one-photon UVA phasor-based hyperspectral light sheet microscopy for the investigation of metabolic activity in mouse embryos. Single excitation simplifies metabolic imaging of FAD and NADH. Moreover, phasor-based hyperspectral detection provides a gain in sensitivity and accuracy in assessing RR for NADH/FAD co-excitation while light sheet microscopy allows for high-speed acquisitions required for rapid 3D imaging. Collectively, this work opens new prospects for the clinical evaluation of metabolic activity in embryology.

References

1. P.N. Hedde, R. Cinco, L. Malacrida, A. Karnaid, E. Gratton, *Communications biology* (2021)
2. P.F. Favreau, J. He, D.A. Gil, D.A. Deming, J. Huisken, M.C. Skala, *Biomedical optics express* (2020)
3. T. Sanchez, M. Venturas, S.A. Aghyami, X. Yang, S. Fraden, D. Sakkas, D.J. Needleman, *Human Reproduction* (2019)