Supplementary Movie 1: Maximum intensity projection renderings of both 3D and 2D unmixed results of a 4-color zebrafish provide visual comparison between the HyU and LU methods, highlighting the improvements for HyU in both the 3D projections and 2D slices. The tetra-labeled specimen used here is Gt(cltca-citrine);Tg(ubiq:lyn-tdTomato; ubiq:Lifeact-mRuby;fli1:mKO2) with citrine in green, tdTomato in yellow, mRuby in cyan, and mKO2 in magenta.

Supplementary Movie 2: A time-lapse of both the 3D maximum intensity projection and 2D single z-slice representations of the HyU unmixed results of a 3-color zebrafish demonstrated the feasibility of low-power, high resolution, *in vivo* time-lapse imaging by utilizing HyU in conjunction with hyperspectral imaging. The tri-labeled specimen used here was Gt(cltca-citrine); Tg(fli1:mKO2; kdrl:mCherry) with citrine in cyan, mKO2 in yellow, and mCherry in magenta.

Supplementary Movie 3: A collage of five 3D and 2D, two-channel renderings of different pairs of labels delineates the multiplexing capabilities of the HyU and its ability to allow simultaneous identification and unmixing of five intrinsic and four extrinsic markers within a zebrafish specimen. The five intrinsic labels are NADH free (yellow), NADH bound (red), Retinol (magenta), Retinoic Acid (cyan), and a mixture of remaining intrinsic signals coined as just autofluorescence (blue). The four intrinsic signals are the familiar Gt(cltca-citrine) in green, Tg(ubiq:lyn-tdTomato) in yellow, Tg(ubiq:Lifeact-mRuby) in cyan, and Tg(fli:mKO2) in magenta.