Haploid Differentiation in Maize Kernels Based on Fluorescence Imaging

Brett W. Boote[†], Daniel J. Freppon[†], Gerald N. De La Fuente[‡], Thomas Lübberstedt[‡], Basil J. Nikolau,[§] Emily A. Smith[†]*

Affiliations and address

*esmith1@iastate.edu

[†] The Ames Laboratory, U.S. Department of Energy, and Department of Chemistry, Iowa State University, Ames, Iowa 50011-3111, United States

[‡] Department of Agronomy, Iowa State University, Ames, Iowa 50011, United States

§ Department of Biochemistry, Biophysics, and Molecular Biology and Center for Metabolic Biology, Iowa State University, Ames, Iowa 50011, United States

Abstract

A new fluorescence-based method for inbred haploid differentiation in maize kernels was developed by utilizing the *R1-nj* color marker in combination with fluorescence micro-spectroscopy and imaging. Seven inbred lines with varying *R1-nj* expression were used in this study. The fluorescence response of the diploid kernels at the embryonic dye spot was shown to simultaneously exhibit lower intensity and occur at a higher wavelength than the fluorescence of the dye-lacking haploid embryos. Intensity and area thresholds were applied to fluorescence images to sort the haploids from mixed sample populations, and sorting efficiencies of greater than 80% were achieved in all seven inbred lines (with values greater than 90% for five lines). The potential for high throughput sorting when fluorescence imaging is combined with existing technologies for seed handling as well as high sorting efficiency may make fluorescence a viable and promising alternative to current sorting methods for some inbred lines.

Keywords maize-haploid-diploid-fluorescence-sorting

Supporting Information

Table S1. Maximum fluorescence intensities of 10 diploid and haploid '78371A' kernels analyzed at the embryo, showing much lower fluorescence in the diploid seeds. The excitation wavelength was 532 nm operating at 50 mW. All acquisitions were 50 ms.

	Fluorescence Intensity (arbitrary units)	
Kernel	**Diploid**	**Haploid**
1	8489	43277
2	18156	62848
3	3101	49500
4	12992	57001
5	7149	58750
6	7208	58299
7	13188	52703
8	10470	58709
9	7285	63170
10	9264	56457
Average	1.0×10^4	5.6×10^4
St. Dev.	0.4×10^4	0.6×10^4

	Diploid			Haploid		
Line	Kernel	DNA Marker	Visual	Kernel	DNA Marker	Visual
'MS198'	1	D	D	1	H	H
	2	D	D	2	NA	H
	3	D	D	3	NA	H
	4	D	D	4	H	H
	5	D	D	5	H	H
	6	D	D	6	H	H
	7	D	D	7	NA	H
	8	D	D	8	NA	H
	9	D	D	9	NA	H
	10	D	D	10	H	H
'PHR36'	1	D	D	1	H	H
	2	D	D	2	H	H
	3	D	D	3	H	H
	4	D	D	4	NA	H
	5	D	D	5	H	H
	6	D	D	6	H	H
	7	D	D	7	H	H
	8	D	D	8	H	H
	9	D	D	9	H	H
	10	D	D	10	H	H
'PHT77'	1	D	D	1	D	H
	2	D	D	2	D	H
	3	D	D	3	NA	H
	4	NA	D	4	D	H
	5	D	D	5	NA	H
	6	NA	D	6	D	H
	7	D	D	7	D	H
	8	D	D	8	D	H
	9	D	D	9	H	H
	10	D	Contamn	10	H	H
'PHK35'	1	NA	D	1	H	H
	2	D	D	2	D	H
	3	D	D	3	H	H
	4	D	D	4	NA	H
	5	D	D	5	H	H
	6	D	D	6	D	H
	7	D	D	7	H	H
	8	D	D	8	H	H
	9	D	D	9	D	H
	10	D	D	10	H	H
'PHB47'	1	D	D	1	H	H
	2	D	D	2	H	H
	3	D	D	3	H	H
	4	D	D	4	H	H
	5	D	D	5	H	H
	6	D	D	6	H	H

	7	D	D	7	H	H
	8	D	D	8	H	H
	9	D	D	9	H	H
	10	D	D	10	H	H
'NK792'	1	D	D	1	NA	H
	2	D	D	2	D	D
	3	D	D	3	NA	H
	4	D	D	4	D	D
	5	D	D	5	NA	D
	6	D	D	6	NA	D
	7	D	D	7	D	H
	8	D	D	8	D	D
	9	D	D	9	H	D
	10	D	D	10	D	H

Table S2. DNA marker analysis of available inbred lines studied, compared to the original visual ploidy classification. Here H = haploid, D = diploid, NA = nonviable seed (did not germinate, classified as haploid, shown in blue text), Contamn = outcross contaminant (where no *R1-nj* expression is seen, green text). In most cases, the visual sort is good, but difficult lines such as PHT77 and NK792 show many visual errors. Differences between visual and genetic methods are highlighted with red text.

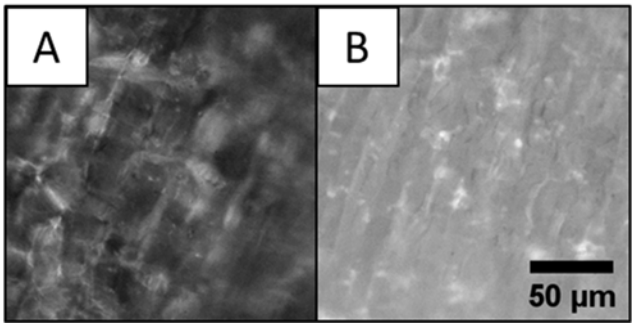

Figure S1. Fluorescence images of (A) diploid and (B) haploid embryo cells from '78371A'. The images were collected using a Nikon microscope fitted with a 10× (0.30 NA) objective using 540 ± 15 nm excitation and 620 ± 25 nm emission filters. The images have been autoscaled for maximum black/white contrast. The overall intensity profile of the diploid kernel is much lower than the haploid kernel. Full-image fluorescence intensity averages were calculated using ImageJ to quantify the difference in diploid and haploid response, and the diploids showed an 85% reduction in overall fluorescence intensity.

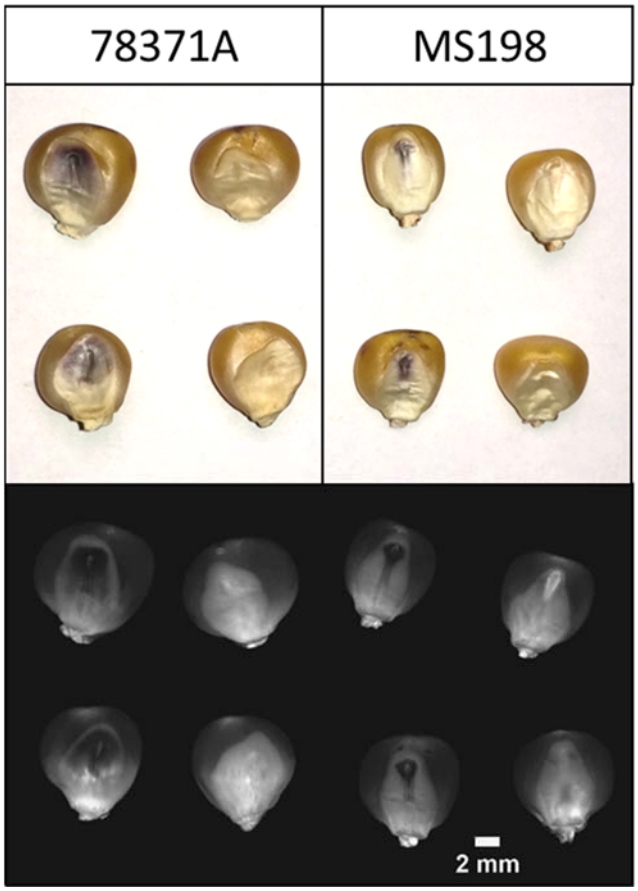

Figure S2. Optical images and corresponding fluorescence images of representative diploid and haploid maize kernels showing the local fluorescence intensity within the germ. The dye spot size and shape varies with each lot studied and there are also small variations from kernel-to-kernel. Note the location of the dye corresponds to a low local fluorescence intensity.

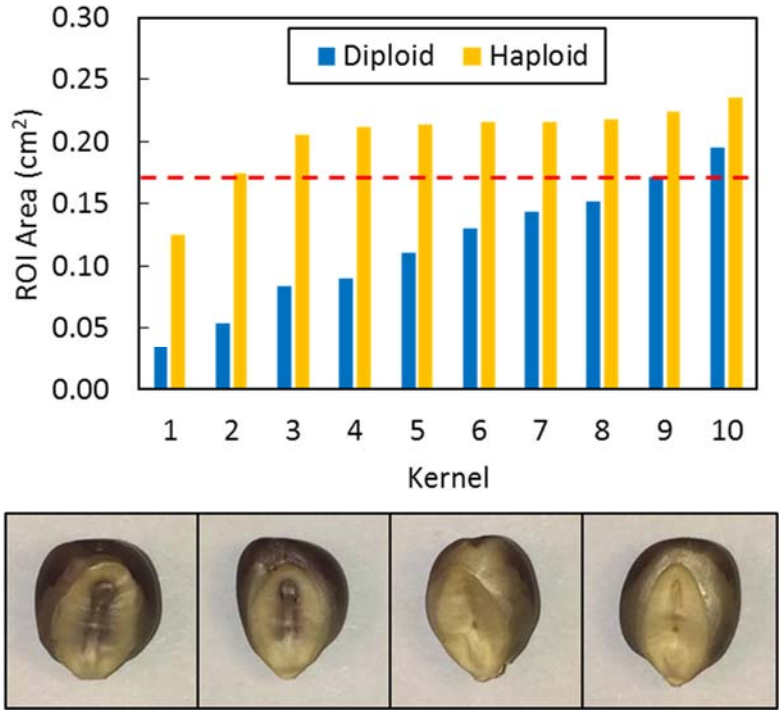

Figure S3. Sorting histogram and two diploid (left) and two haploid (right) kernel images for 'PHR36'. The threshold identified 9 of 10 haploids with 1 false positive.

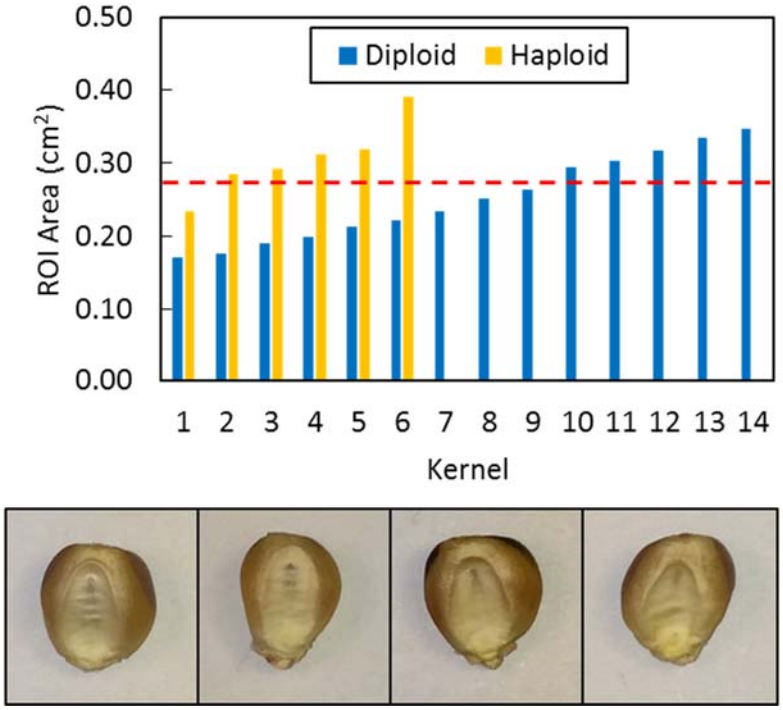

Figure S4. Sorting histogram and two diploid (left) and two haploid (right) kernel images for 'PHT77'. The threshold identified 5 of 6 haploids with 5 false positives. In the images it is clear this is a difficult line to sort visually; DNA marker analysis showed several visually misclassified kernels. This is what led to the uneven population of haploid/diploid kernels. The *R1-nj* expression produces a small, hard to see spot in this line, and the haploids have slightly colored embryos as well.

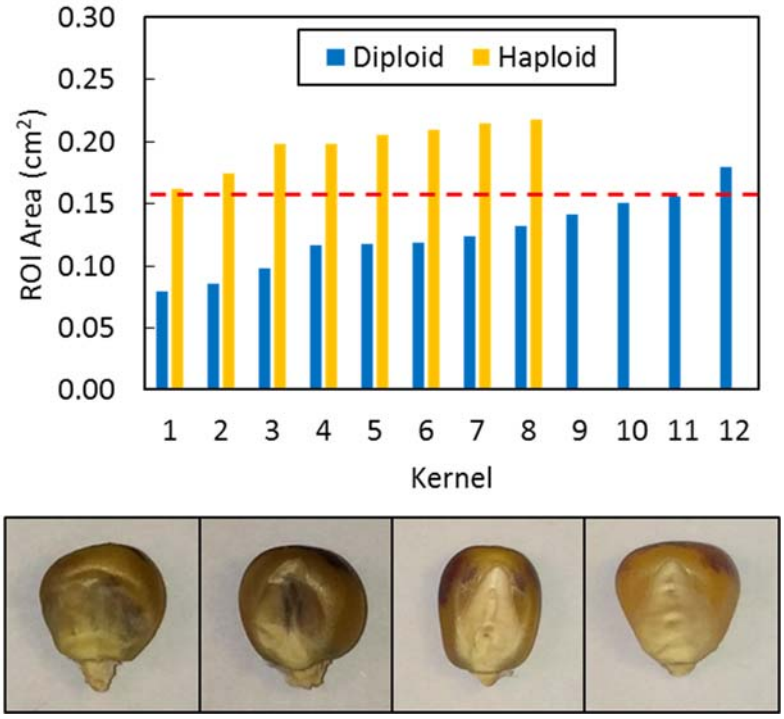

Figure S5. Sorting histogram and two diploid (left) and two haploid (right) kernel images for 'PHK35'. The threshold identified 8 of 8 haploids with 1 false positive.

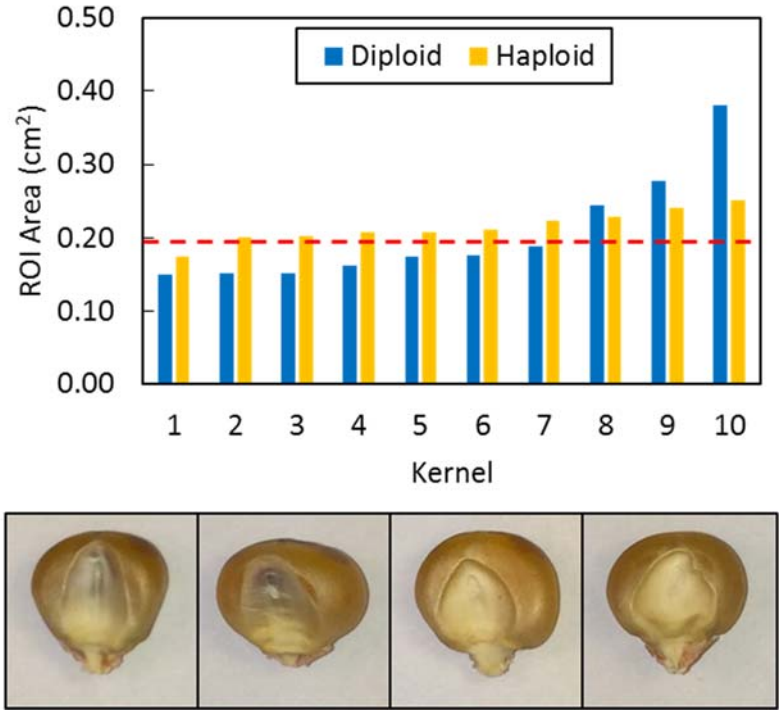

Figure S6. Sorting histogram and two diploid (left) and two haploid (right) kernel images for 'PHB47'. The threshold identified 9 of 10 haploids with 3 false positives.

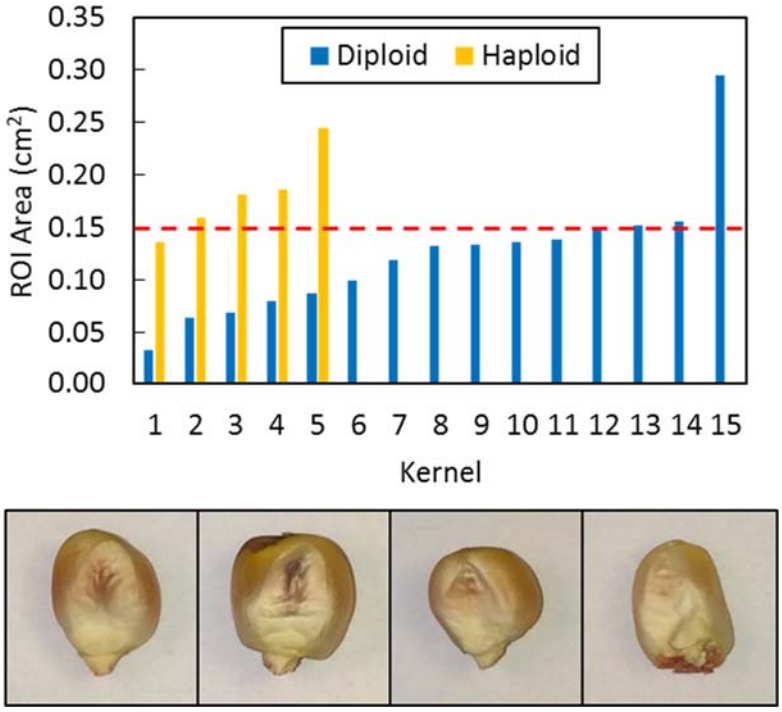

Figure S7. Sorting histogram and two diploid (left) and two haploid (right) kernel images for 'NK792'. The threshold identified 4 of 5 haploids with 3 false positives. In the images it is clear this is another difficult line to sort visually; DNA marker analysis showed several visually misclassified kernels. This is what led to the uneven population of haploid/diploid kernels. A rose colored embryo seems to obscure the *R1-nj* expression in the diploids.

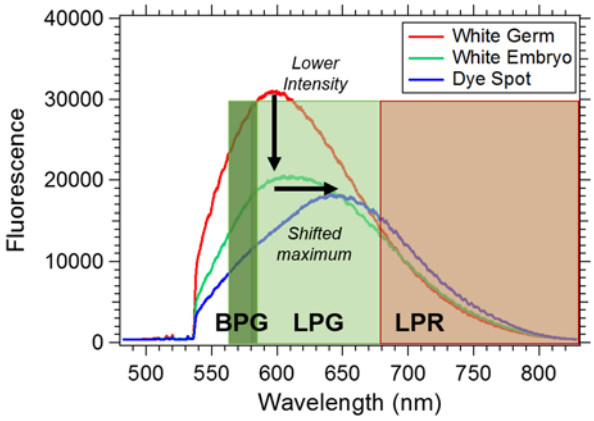

Figure S8. Plot of selected fluorescence spectra from a diploid '78371A' kernel with the three filters used for the comparison (575-nm long pass, LPG; 570 ± 10-nm band pass, BPG; 665-nm long pass, LPR). The BPG filter produces the best sort due to both the lower intensity and red-shifted maximum wavelength of the fluorescence at the dye spot. These representative spectra were selected from a line scan of the kernel as shown in Figure 2.