Evaluation of Highlighter Pens as Cheap and Cheerful Samples for Microscope Calibration and Performance Testing

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MAX-PLANCK-GESELLSCHAFT

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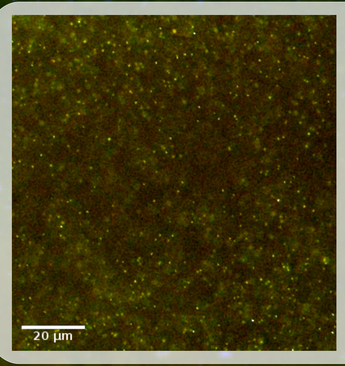
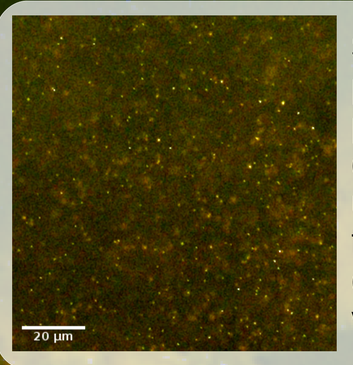
INTRODUCTION:

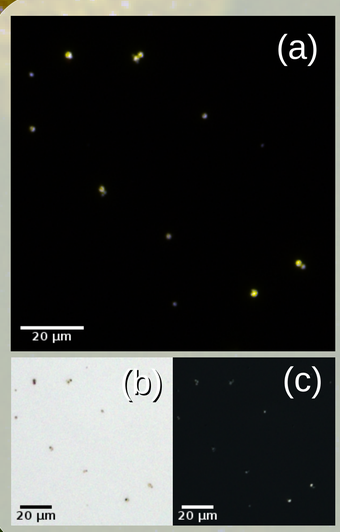
The bright appearance of highlighter pens is usually connected to the presence of some fluorescent component within the highlighter fluid. Those components can be dissolved fluorophores or a suspension of fluorescent particles. Here we evaluate several highlighter pens and their fluids with regard to their usefulness in the context of microscope performance evaluation.



Zebra 78105 (Mildliner)™ pens contain spherical sub-resolution fluorescent particles of uniform size.

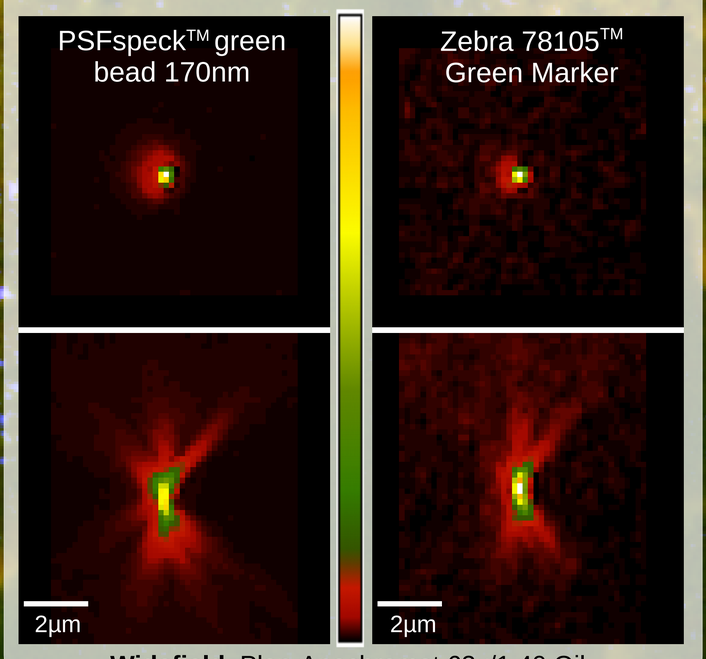


Stabilo Pen 68 neon™ pens contain spherical particles which are quite bright and of non-uniform size. Apart from fluorescence also darkfield can be used to visualise the particles.



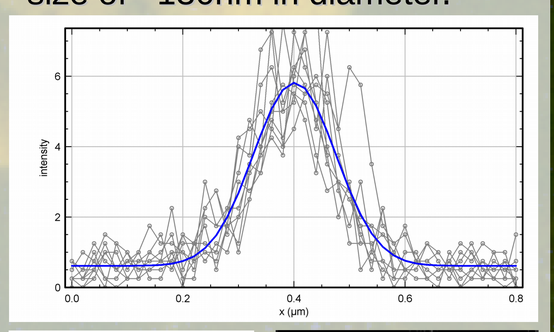
Pilot Frixion light and light soft™ pens contain bright, comparably large and quite irregular particles, usually with a mixture of fluorophores on a single particle (a), they are also easily detectable with brightfield (b), DIC (c) and darkfield (not shown).

Zebra 78105™ particles cross sections show same features as commercial beads used for microscope performance control.



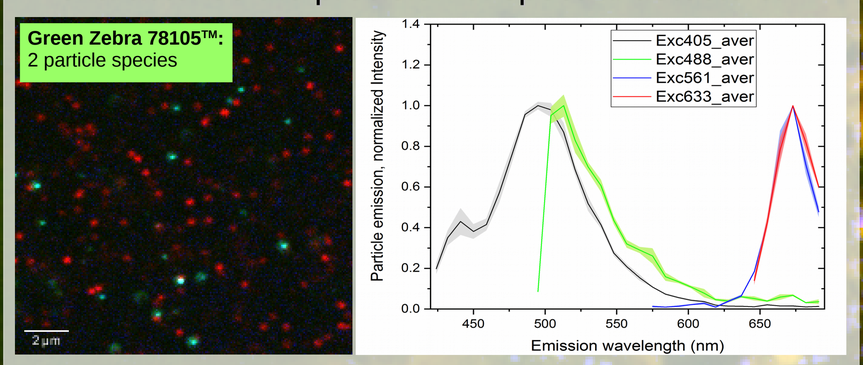
Widefield, Plan-Apochromat 63x/1,40 Oil
filter 450-490/495/500-550

Zebra 78105™ orange beads are stable enough to withstand STED conditions and exhibit a size of ~130nm in diameter.

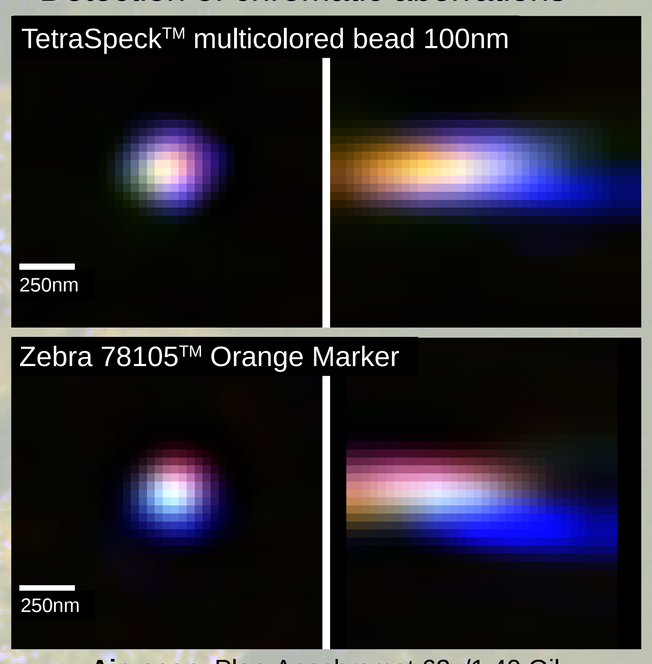


STED, Plan-Apo 100x/1.46 Oil, ex594,
depl775 @ 1250mW

Spectra of **Zebra 78105™** show a strong reproducibility and their shape is ideally suited to characterise the precision of spectral detectors.



Detection of chromatic aberrations



Airyscan, Plan-Apochromat 63x/1,40 Oil
Ch1 (blue) ex405, Ch2 (green) ex488, Ch3 (red) ex561

CONCLUSIONS:

Fluids of highlighter pens and the particles therein can be used as a cost effective alternative to classic beads for a wide range of quality control tasks and all basic (and some advanced) maintenance measurements can be performed with the aid of these. From the pens used in our study we found the Zebra 78105 (Mildliner)™ pens to be the most versatile one.

please find more information at:
http://webdav.tuebingen.mpg.de/LM/

Which brand & model of pen can be used for what

brand/model	*type*	*Parfocality/ Parcentricity/ stage precision*	*PSF recording*	*spectral registration*	*spectral calibration*	*field flatness*	*illumination homogeneity*
ZebraPen 78105 Mildliner pastel	particle	fluorescence only	**yes**	**yes**	**yes**	sort of	limited
Pilot Frixion light/light soft	particle	**yes**	no	no	no	sort of	no
Stabilo pen 68 neon	particle	fluorescence only	no	-/-	-/-	sort of	no
Stabilo BOSS neon	particle	no	no	no	no	sort of	limited
Pelikan 438	solution	no	no	no	no	no	**yes**

to be tested

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