This MICCAI paper is the Open Access version, provided by the MICCAI Society. It is identical to the accepted version, except for the format and this watermark; the final published version is available on SpringerLink.

Supplementary Material
Investigation of Factors Contributing to Domain Generalization in Single-Shot Autofocus

A. The substitution holds within every training source

Table S1 reports the out-of-domain MAE for every combination of input, augmentation preset and training source, instead of averaging over the source. The input × augmentation × source interaction is significant ($\chi^2_{20} = 122.2$, $p < 10^{-15}$, Type III joint test): the size of the input effect differs by source.

Under base augmentation every alternative input has a lower error than RGB in all six sources (24 of 24 contrasts negative, 14 individually significant). Under the photometric preset the five inputs lie within 0.41 μm of one another in every source and no contrast is significant.

Table S1. Out-of-domain estimated marginal MAE (microns, 95% CI) by training source, input and augmentation preset, from a mixed model with the full input × augmentation × source interaction and the same random effects as the main analysis.

Training source	RGB	gray	FFT	gray+FFT	DWT
Base augmentation (geometric only)					
ZStack_HE	4.22 (3.6, 4.8)	3.97 (3.3, 4.6)	3.90 (3.3, 4.5)	4.12 (3.5, 4.7)	3.56 (2.9, 4.2)
ZStack_IHC	6.33 (5.7, 6.9)	5.76 (5.1, 6.4)	3.62 (3.0, 4.2)	4.20 (3.6, 4.8)	3.29 (2.7, 3.9)
HE-MultiOrgan	5.17 (4.6, 5.8)	4.50 (3.9, 5.1)	4.35 (3.7, 5.0)	4.45 (3.8, 5.1)	5.01 (4.4, 5.6)
AgNOR	5.01 (4.4, 5.6)	3.83 (3.2, 4.4)	4.14 (3.5, 4.7)	3.79 (3.2, 4.4)	3.96 (3.3, 4.6)
IHC-MultiStain	11.34 (10.7, 12.0)	6.60 (6.0, 7.2)	5.23 (4.6, 5.8)	5.08 (4.5, 5.7)	6.31 (5.7, 6.9)
Jiang2018	8.17 (7.6, 8.8)	5.22 (4.6, 5.8)	3.95 (3.3, 4.6)	3.93 (3.3, 4.5)	3.39 (2.8, 4.0)
Photometric augmentation					
ZStack_HE	3.50 (2.9, 4.1)	3.45 (2.8, 4.1)	3.88 (3.3, 4.5)	3.90 (3.3, 4.5)	3.31 (2.7, 3.9)
ZStack_IHC	3.94 (3.3, 4.6)	3.54 (2.9, 4.2)	3.68 (3.1, 4.3)	3.71 (3.1, 4.3)	3.64 (3.0, 4.3)
HE-MultiOrgan	4.65 (4.0, 5.3)	4.77 (4.2, 5.4)	4.55 (3.9, 5.2)	4.68 (4.1, 5.3)	4.63 (4.0, 5.3)
AgNOR	3.73 (3.1, 4.3)	3.91 (3.3, 4.5)	3.70 (3.1, 4.3)	4.01 (3.4, 4.6)	4.04 (3.4, 4.7)
IHC-MultiStain	6.20 (5.6, 6.8)	6.04 (5.4, 6.7)	6.24 (5.6, 6.9)	6.04 (5.4, 6.7)	6.07 (5.5, 6.7)
Jiang2018	3.51 (2.9, 4.1)	3.49 (2.9, 4.1)	3.74 (3.1, 4.4)	3.72 (3.1, 4.3)	3.30 (2.7, 3.9)

B. The substitution holds on a second backbone

Table S2 compares the ResNet-18 means of Table 2 of the main paper with an EfficientNet-B0 replication of the same sweep. The substitution holds on both backbones. Under base augmentation, channel collapse accounts for most of the

gain over RGB (1.73 μm on ResNet-18, $p < 10^{-12}$; 1.10 μm on EfficientNet-B0, $p = 0.024$), and under the photometric preset no contrast against grayscale is significant (each $p = 1$). The smaller transform-over-grayscale component reaches significance only on ResNet-18 (0.72–0.78 μm, each $p < 0.012$); on EfficientNet-B0 it measures 0.54–0.70 μm and does not (each $p > 0.32$). On both backbones it vanishes under photometric augmentation.

Table S2. Out-of-domain estimated marginal MAE (microns, 95% CI) by input, augmentation preset and backbone. Means are averaged over training source and estimated from separate mixed models per backbone.

	ResNet-18		EfficientNet-B0	
Input	Base	Photometric	Base	Photometric
RGB	6.71 (6.33, 7.09)	4.26 (3.88, 4.63)	6.15 (5.57, 6.74)	4.48 (3.89, 5.07)
gray	4.98 (4.60, 5.36)	4.20 (3.82, 4.58)	5.05 (4.47, 5.64)	4.25 (3.67, 4.84)
FFT	4.20 (3.82, 4.57)	4.30 (3.92, 4.68)	4.36 (3.77, 4.94)	4.32 (3.74, 4.91)
gray+FFT	4.26 (3.88, 4.64)	4.34 (3.97, 4.72)	4.37 (3.78, 4.95)	4.20 (3.62, 4.79)
DWT	4.25 (3.87, 4.63)	4.16 (3.79, 4.54)	4.51 (3.92, 5.10)	4.23 (3.65, 4.82)