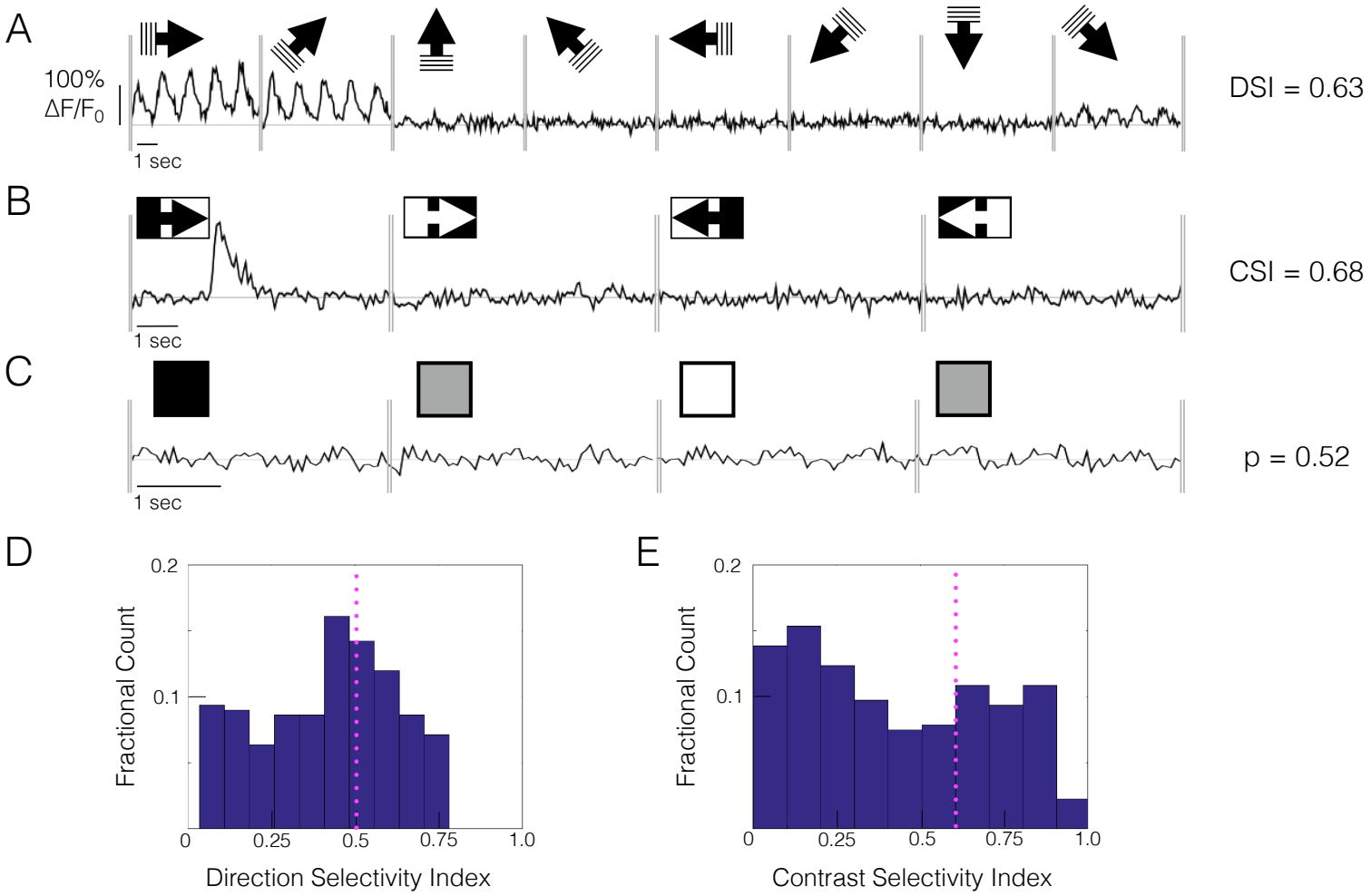
A
100%
ΔF/F0
1 sec
DSI = 0.63
B
1 sec
CSI = 0.68
C
1 sec
p = 0.52
D
Fractional Count
0.2
0.1
0
0.25
0.5
0.75
1.0
Direction Selectivity Index
E
Fractional Count
0.2
0.1
0
0.25
0.5
0.75
1.0
Contrast Selectivity Index

Figure S1

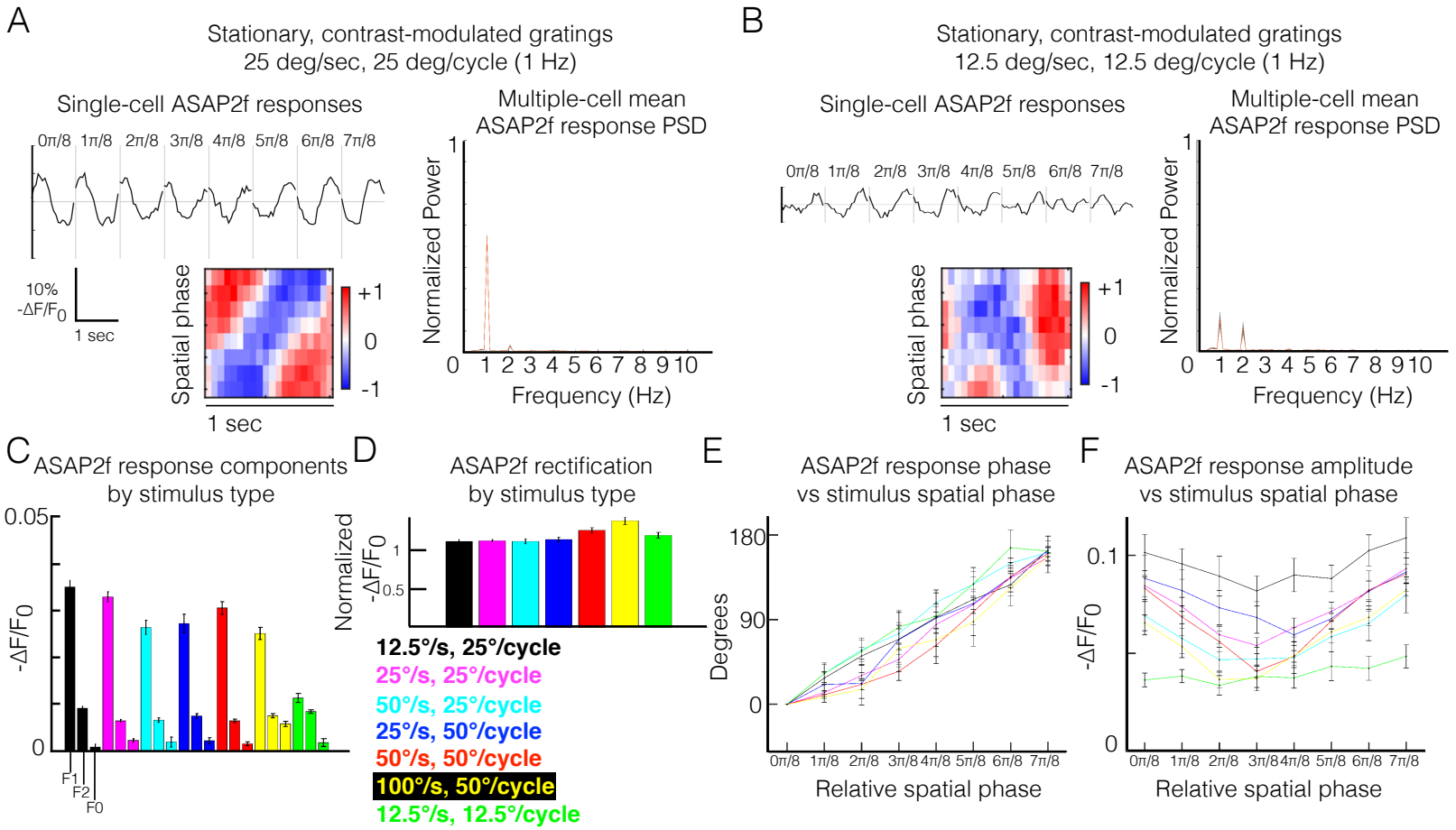
A
Stationary, contrast-modulated gratings
25 deg/sec, 25 deg/cycle (1 Hz)
Single-cell ASAP2f responses
0π/8 1π/8 2π/8 3π/8 4π/8 5π/8 6π/8 7π/8
10%
-ΔF/F0
1 sec
Spatial phase
+1
0
-1
1 sec
Multiple-cell mean
ASAP2f response PSD
Normalized Power
1
0 1 2 3 4 5 6 7 8 9 10
Frequency (Hz)
B
Stationary, contrast-modulated gratings
12.5 deg/sec, 12.5 deg/cycle (1 Hz)
Single-cell ASAP2f responses
0π/8 1π/8 2π/8 3π/8 4π/8 5π/8 6π/8 7π/8
Spatial phase
+1
0
-1
1 sec
Multiple-cell mean
ASAP2f response PSD
Normalized Power
1
0 1 2 3 4 5 6 7 8 9 10
Frequency (Hz)
C
ASAP2f response components
by stimulus type
0.05
-ΔF/F0
0
F1
F2
F0
D
ASAP2f rectification
by stimulus type
Normalized
-ΔF/F0
1
0.5
12.5°/s, 25°/cycle
25°/s, 25°/cycle
50°/s, 25°/cycle
25°/s, 50°/cycle
50°/s, 50°/cycle
100°/s, 50°/cycle
12.5°/s, 12.5°/cycle
E
ASAP2f response phase
vs stimulus spatial phase
Degrees
180
90
0
0π/8 1π/8 2π/8 3π/8 4π/8 5π/8 6π/8 7π/8
Relative spatial phase
F
ASAP2f response amplitude
vs stimulus spatial phase
-ΔF/F0
0.1
0
0π/8 1π/8 2π/8 3π/8 4π/8 5π/8 6π/8 7π/8
Relative spatial phase

Figure S2

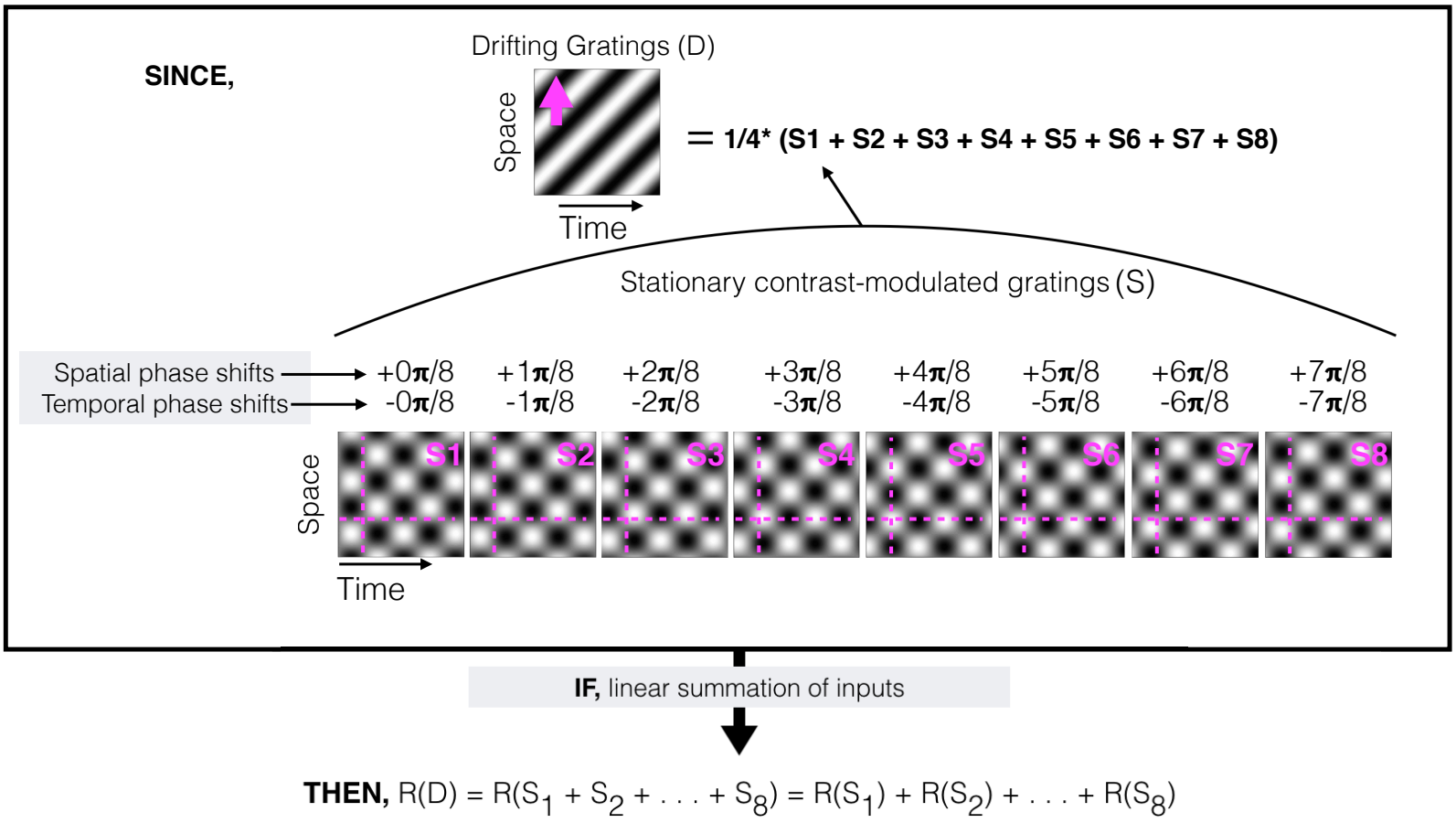
SINCE,
Drifting Gratings (D)
Space
Time
= 1/4* (S1 + S2 + S3 + S4 + S5 + S6 + S7 + S8)
Stationary contrast-modulated gratings (S)
Spatial phase shifts
Temporal phase shifts
+0π/8 +1π/8 +2π/8 +3π/8 +4π/8 +5π/8 +6π/8 +7π/8
-0π/8 -1π/8 -2π/8 -3π/8 -4π/8 -5π/8 -6π/8 -7π/8
S1 S2 S3 S4 S5 S6 S7 S8
Space
Time
IF, linear summation of inputs
THEN, R(D) = R(S1 + S2 + . . . + S8) = R(S1) + R(S2) + . . . + R(S8)

Figure S3

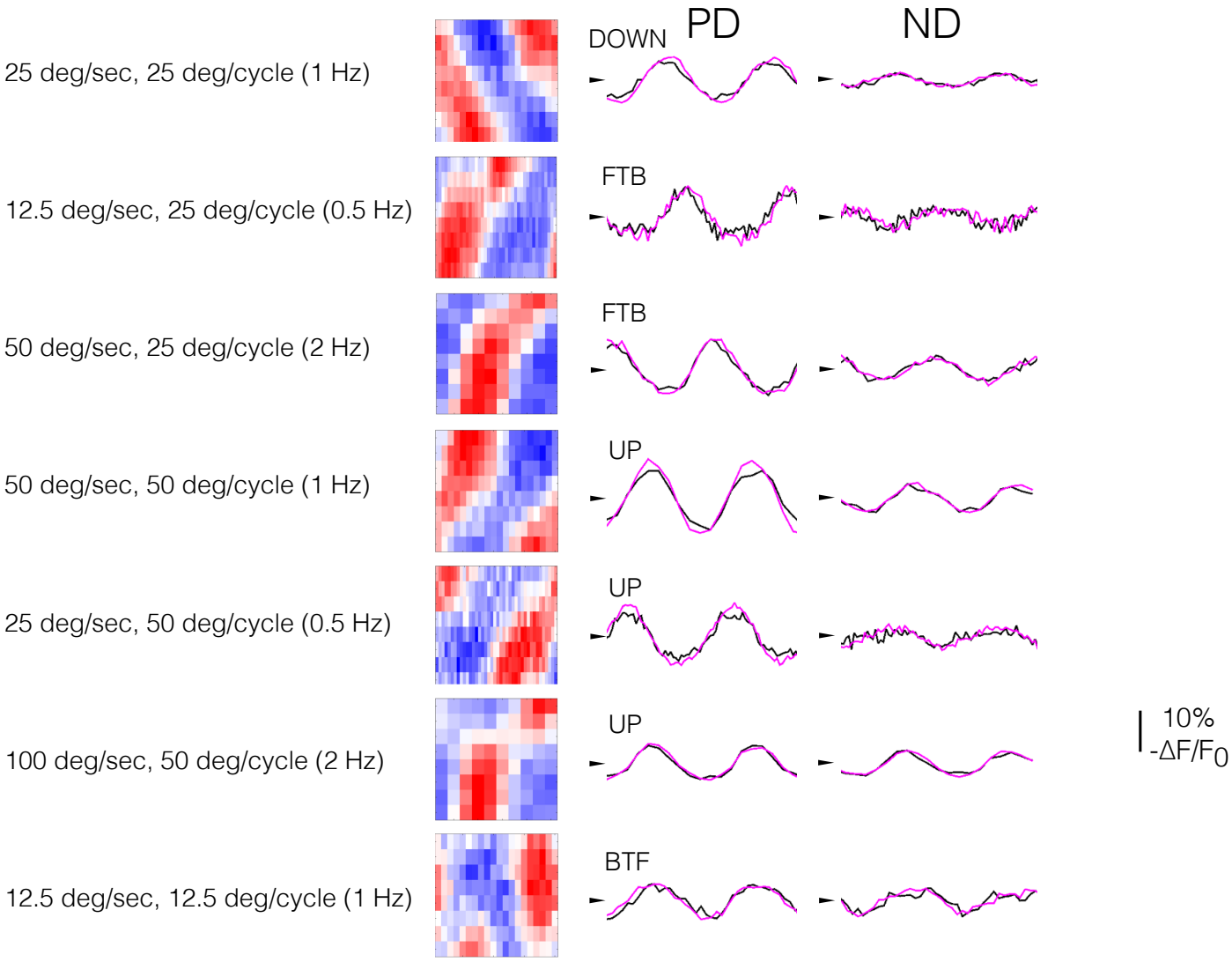
PD
ND
25 deg/sec, 25 deg/cycle (1 Hz)
DOWN
12.5 deg/sec, 25 deg/cycle (0.5 Hz)
FTB
50 deg/sec, 25 deg/cycle (2 Hz)
FTB
50 deg/sec, 50 deg/cycle (1 Hz)
UP
25 deg/sec, 50 deg/cycle (0.5 Hz)
UP
100 deg/sec, 50 deg/cycle (2 Hz)
UP
12.5 deg/sec, 12.5 deg/cycle (1 Hz)
BTF
10%
-ΔF/F0

Figure S4

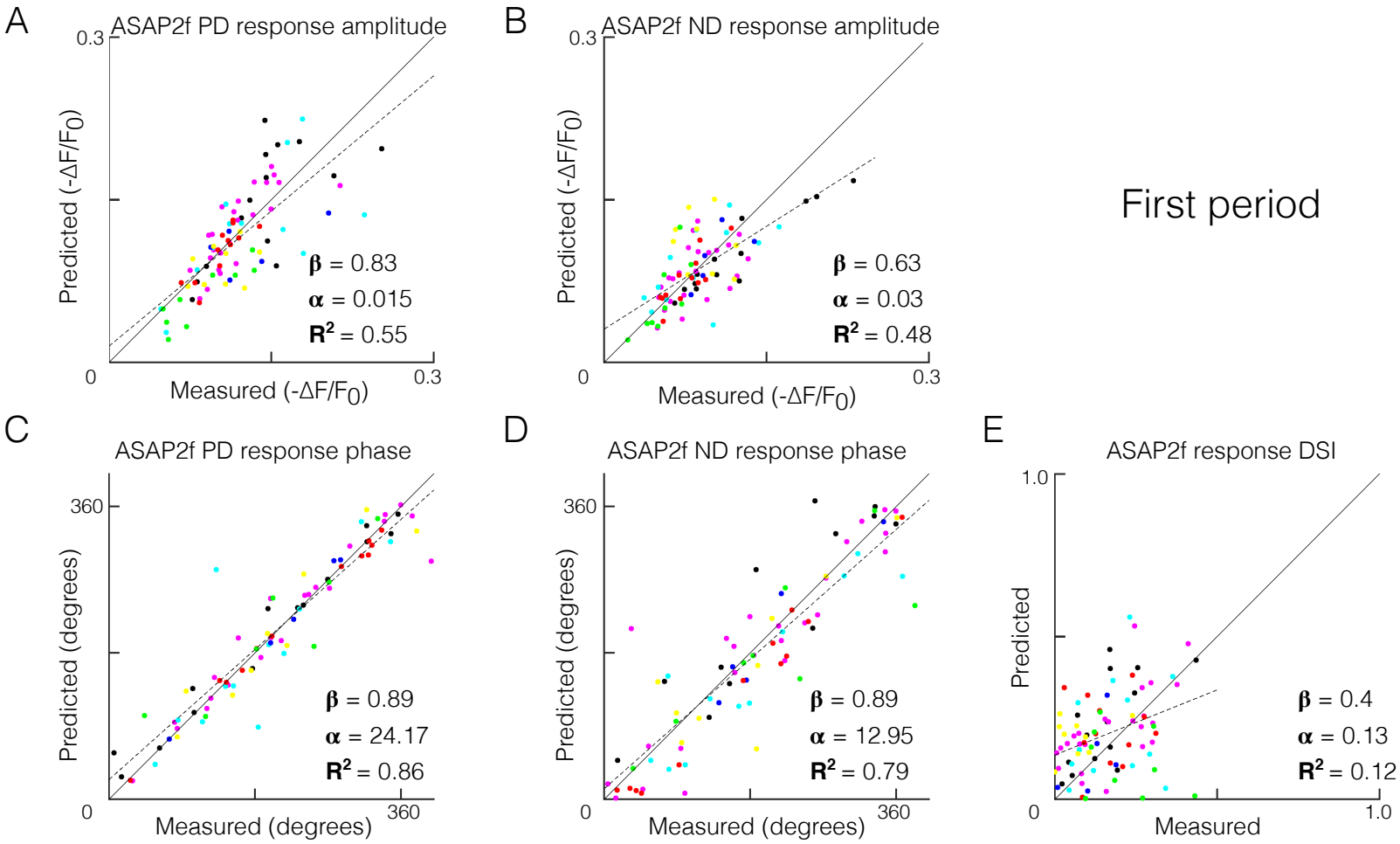
A
ASAP2f PD response amplitude
Predicted (-ΔF/F0)
Measured (-ΔF/F0)
0.3
0
β = 0.83
α = 0.015
R2 = 0.55
B
ASAP2f ND response amplitude
β = 0.63
α = 0.03
R2 = 0.48
First period
C
ASAP2f PD response phase
Predicted (degrees)
Measured (degrees)
360
β = 0.89
α = 24.17
R2 = 0.86
D
ASAP2f ND response phase
β = 0.89
α = 12.95
R2 = 0.79
E
ASAP2f response DSI
Predicted
Measured
1.0
β = 0.4
α = 0.13
R2 = 0.12

Figure S5

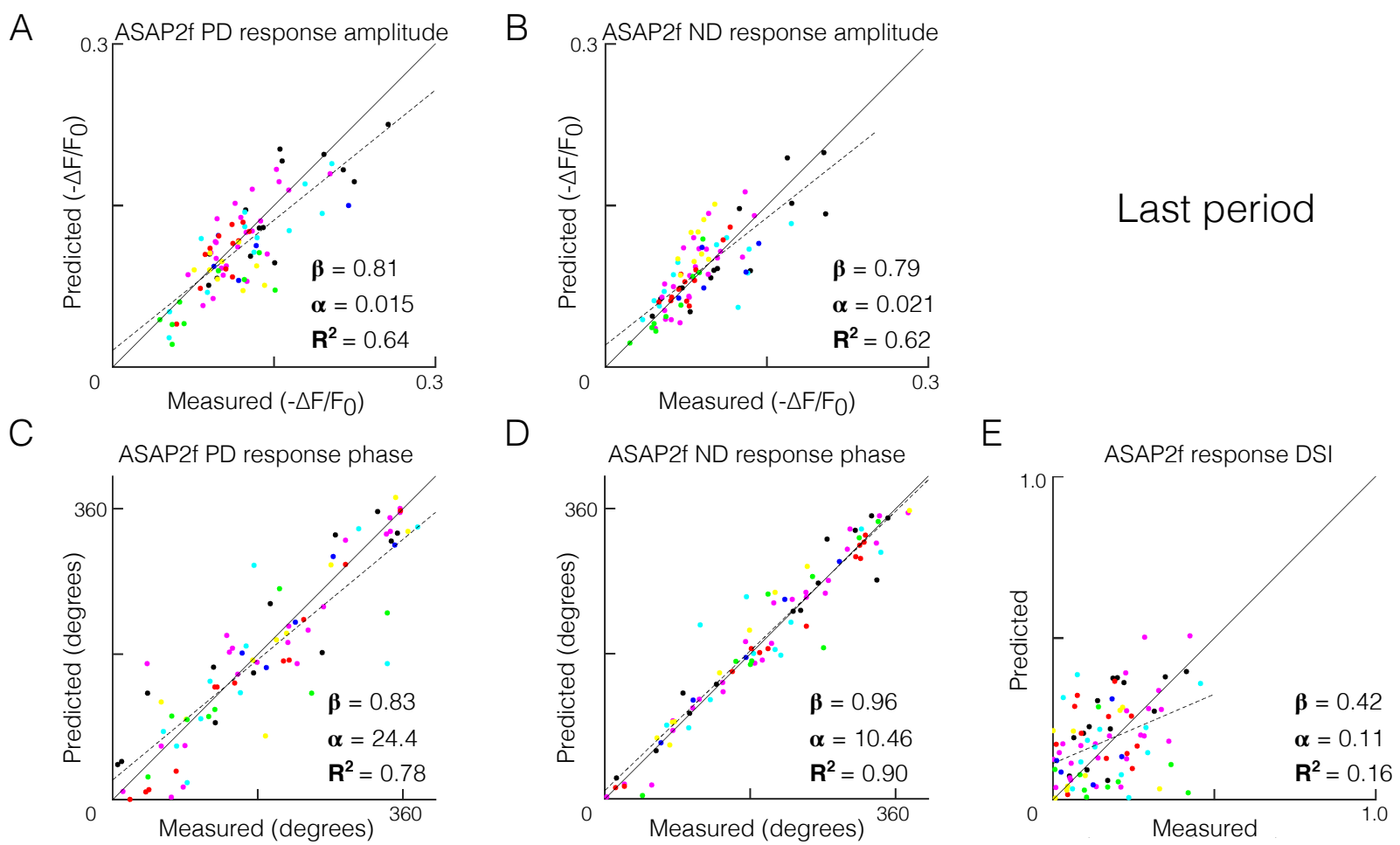
A
ASAP2f PD response amplitude
Predicted (-ΔF/F0)
Measured (-ΔF/F0)
0.3
0
β = 0.81
α = 0.015
R2 = 0.64
B
ASAP2f ND response amplitude
β = 0.79
α = 0.021
R2 = 0.62
Last period
C
ASAP2f PD response phase
Predicted (degrees)
Measured (degrees)
360
β = 0.83
α = 24.4
R2 = 0.78
D
ASAP2f ND response phase
β = 0.96
α = 10.46
R2 = 0.90
E
ASAP2f response DSI
Predicted
Measured
1.0
β = 0.42
α = 0.11
R2 = 0.16

Figure S6

SUPPLEMENTAL FIGURES TITLES AND LEGENDS

Figure S1. Related to Figures 1-4, S2, and S4-S6: Calcium signals for ROI selection
(A-C) jRGECO1a response timecourses to moving 1Hz sinusoidal gratings at 30°/cycle in eight equally spaced directions (A), moving light and dark edges in PD and ND (B), and full-field contrast increments and decrements (C). Responses to these three stimuli were used to determine whether ROIs represented T5 single cells that were suitable for further analysis (see methods). Same single cell as in Figure 3A-E.
(D, E) Distribution of direction selectivity indices (D) and contrast-selectivity indices (E) for an initial set of ROIs that were functionally isolated from sparse T5 expression patterns. 283 ROIs from 28 flies. Magenta dotted lines indicate thresholds (DSI: 0.5, CSI: 0.6), below which ROIs were not considered single cells, and were excluded from further analysis.

Figure S2: Voltage responses to stationary, sinusoidally contrast-modulated gratings demonstrate linear filtering across a range of spatial and temporal frequencies
(A) Top left, ASAP2f response timecourses to stationary, sinusoidally contrast-modulated gratings at 1 Hz, 25°/cycle, at eight equally-spaced spatial phases. For each spatial phase of the stimulus, the mean response to a single stimulus cycle is shown. Bottom left, spacetime plot of responses to stationary gratings above; each row represents the normalized mean response to one stimulus cycle for each spatial phase of the stimulus. Colorbar and normalization as in Figure 1D. Right, normalized ASAP2f response power spectral density, averaged across all cells responding to gratings at 1 Hz, 25°/cycle, and averaged across all eight spatial phases. Shading denotes +/- 1 SEM. Note the dominance of the F1 component (in this case, the 1 Hz component).
(B) Top left and bottom left, same as in panel A, but for a different cell, responding to stationary, sinusoidally contrast-modulated gratings at 1 Hz, 12.5°/cycle. Note the 2 Hz signal (doubling) at the two rightmost spatial phases. Right, same as panel A (right), but for all cells responding to 1 Hz gratings at 12.5°/cycle. Note the prominence of the F2 component (in this case, 2 Hz component), relative to the F1 component.
Panels C-F present metrics averaged across all cells (same set of cells as in Figure 2C and Figure 4) responding to a particular type of grating, each having a unique spatial and temporal frequency combination, and each represented by a different color (same color coding as in Figure 2C and Figure 4). Panels C and D represent responses averaged across all spatial phases. For panels C-F, error bars denote +/- 1 SEM.
(C) Quantification of ASAP2f responses to stationary, sinusoidally contrast-modulated gratings. For each grating type (color). Bars (from left to right) represent the response F1 component, F2 component, and F0 component.
(D) For each grating type (color), ASAP2f responses display modest depolarizing rectification. Rectification = max/abs(min), so a value of 1.0 indicates no rectification, and values greater than 1.0 indicate a bias towards depolarization.
(E)-(F) Relative temporal phase (E) and amplitude (F) of the ASAP2f response vs stimulus spatial phase, for each grating type (color). For panel E, temporal phase was quantified as the phase of the F1 component of the response; temporal phase was normalized, then registered across cells by placing the response of maximum amplitude at the rightmost spatial phase. For panel F, amplitude (max minus min) was also registered across cells in the same way.

Figure S3. Related to Figures 3-4, and S4-6: Spacetime synthesis of moving gratings from stationary gratings
Top, moving sinusoidal gratings are equivalent to the scaled sum of spatially and temporally phase-shifted stationary sinusoidally contrast-modulated gratings. In spacetime, a moving

grating moving at a constant velocity in one direction is represented as a “barber pole”, or periodic tilted stripes. Motion in the opposite direction corresponds to a reflection of the barber pole across the space axis that reverses the tilt (not pictured). A stationary contrast-modulated grating is represented as a “checkerboard”, with sinusoidal contrast modulation over space and time. Spatial and temporal phase shifts correspond to translations of the checkerboard along the space and time axes, respectively. To synthesize a moving grating, stationary gratings must be shifted proportionally in spatial and temporal phase, then summed and scaled. To synthesize gratings moving in the opposite direction, the same spatial phase shifts are applied, but the temporal phase shifts are reversed (not pictured). Magenta dashed lines highlight a single spatial and temporal phase. Bottom, because moving gratings are equal to the scaled sum of phase-shifted stationary contrast-modulated gratings, for a neuron that sums inputs linearly over space, the scaled sum of responses to the stationary gratings will equal the response to a moving grating. In our experiments, the stationary gratings were presented at different spatial phases, but identical temporal phase. Thus, to make the linear prediction, the responses were temporally shifted by an amount proportional to the spatial phase shift of the stimulus, prior to summation (as in Figure 3D).

Figure S4. Related to Figure 4. A linear model predicts T5 voltage responses to moving sinusoidal gratings across a range of spatial and temporal frequencies
For each type of grating represented in Figures 2C, 4, and S2 the voltage response timecourses and predicted timecourses of a representative ROI is shown (right) along with the XT plot of the responses to stationary-contrast modulated gratings used to generate the linear prediction (left). As in Figure 3E-F, black represents responses to drifting sinusoidal gratings in PD and ND, and magenta represents the linear prediction of those responses. For timecourses, the mean measured and predicted responses to two stimulus cycles are presented. Spacetime plots are as in Figure 3, and represent mean responses to a single stimulus cycle. No timescale is given because the timescale varies according to the temporal frequency of the stimulus. All of these cells are part of the dataset depicted in Figures 2C, 4, and S2.

Figure S5: Related to Figure 4. A linear model quantitatively predicts direction-selective responses in T5 during the first presentation of the stimulus
Data is presented and analyzed as in Figure 4, except only responses to the first of eight cycles of gratings are used (for measured and predicted responses). Thus, the linear prediction is constructed only from responses to the first of eight cycles of the gratings (depending on the temporal frequency of the gratings, this corresponds to the first 0.5-2 seconds of the mean response to the 8-second bout). The similarities among this figure, Figure 4, and Figure S6 show that the accuracy of our linear model does not appear to be influenced by adaptation to repeated presentations of the visual stimuli; that is, linearity holds across different timescales. As in Figure 4, dashed lines are least-squares fits to the data. Same set of cells and color coding as in Figures 2C, 4, S2C-F, and S6.

Figure S6: Related to Figure 4. A linear model quantitatively predicts direction-selective responses in T5 during the final presentation of the stimulus
Same as Figure S5, except only responses to the last of eight cycles of gratings are used (for measured and predicted responses). Same set of cells and color coding as in Figures 2C, 4, S2C-F, and S5.