\mathbf{A}	image processing
Adipocyte, see Differentiating cell systems	apoptosis assay, 360-362, 364
AGT, see Alkylguanine-DNA	proliferation assay, 362
alkyltransferase	imaging of plates, 356, 357, 359, 360
Akt	materials, 353, 354, 363
high content screening assays for target	overview, 353
validation	staining
cell line selection, 368, 373	bromodeoxyuridine,
immunoassays of phosphorylated	357–359, 364
proteins, 372, 373–375	Hoechst 33342 counterstaining,
materials, 368	356
multiplexed apoptosis and	YO-PrO-1, 356, 363
proliferation assay, 373–375	transfection, 355, 356
reagent identification, 368–370, 373,	retinal ganglion cells following optic
374	nerve injury, 427, 428
pathway profiling with translocation	Aptamers, fluorescent fixed end-point high
analysis, 411	content screening assays, 147
signaling, 221	ArrayScan(r) VTI, features, 42, 52, 54
translocation inhibitor discovery with	β-Arrestins, protein-fragment
high content screening, 380, 383-	complementation assays for
386	dynamics studies, 228–230
Alkylguanine-DNA alkyltransferase (AGT),	Artificial neural networks, see Machine
SNAP-tag, 181	learning
Anaphase-promoting complex (APC),	Axiovision, image analysis, 64
axonal growth role, 428	_
Angiogenesis, high content screening, 26–	В
28	BacMam, cell engineering, 30
APC, see Anaphase-promoting complex	BDNF, see Brain-derived neurotrophic
Apoptosis	factor
Akt signaling pathway high content	Biocarta, pathway database, 321
screening assay, 373–375	Bioinformatics, see Informatics, high
fluorescent probes, 239, 240	content screening
high content screening cancer studies of	Brain-derived neurotrophic factor (BDNF),
RNA interference knockdown	axonal growth
effects on apoptosis and	role, 428
proliferation	Bromodeoxyuridine, high content screening
cells	cancer studies of RNA interference
seeding on plates, 355, 363	knockdown effects on apoptosis
selection of cell lines, 354, 363	and proliferation
data analysis	cells
apoptosis analysis, 362	seeding on plates, 355, 363
proliferation analysis 362 363	selection of cell lines 354 363

apoptosis analysis, 362 proliferation analysis, 362, 363 image processing apoptosis assay, 360–362, 364 proliferation assay, 362 imaging of plates, 356, 357, 359, 360 materials, 353, 354, 363 overview, 353 staining bromodeoxyuridine, 357–359, 364 Hoechst 33342 counterstaining, 356 YO-PrO-1, 356, 363 transfection, 355, 356 C Caged compounds advantages, 253, 254 batch transfection, 256 cage requirements, 253 delivery to cells, 254, 256 examples and properties, 254–256 small interfering RNA and gene knockdown experiments glyceraldehyde-3-phosphate dehydrogenase knockdown, 258, 259 light-dosage working curve generation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 small molecule probes, 235, 236 small molecule probes, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Cataltyzed reporter enzyme (CARD), fixed end-point high content screening assays, 147 CellCardTM antiproliferative compound assessment in several cell types simultaneously antiproliferative compound assessment in several cell types indiving antiprotiter plate dispensing, 132, 137 tissue culture, 131 overview of system, 129, 130 Cellomics definition, 3 Cellomics definition, 4 ov	data analysis	Catalyzed reporter enzyme deposition
proliferation analysis, 362, 363 image processing apoptosis assay, 360–362, 364 proliferation assay, 362 imaging of plates, 356, 357, 359, 360 materials, 353, 354, 363 overview, 353 staining bromodeoxyuridine, 357–359, 364 Hoechst 33342 counterstaining, 356 YO-PrO-1, 356, 363 transfection, 355, 356 C Caged compounds advantages, 253, 254 batch transfection, 256 cage requirements, 253 delivery to cells, 254, 256 examples and properties, 254–256 small interfering RNA and gene knockdown experiments glyceraldehyde-3-phosphate dehydrogenase knockdown, 258, 259 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260 transfection, 258, 261 UCOM instrumentation for high contents ascrening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme		
image processing apoptosis assay, 360–362, 364 proliferation assay, 362 imaging of plates, 356, 357, 359, 360 materials, 353, 354, 363 overview, 353 staining bromodeoxyuridine, 357–359, 364 Hoechst 33342 counterstaining, 356 YO-PrO-1, 356, 363 transfection, 355, 356 C Caged compounds advantages, 253, 254 batch transfection, 256 cage requirements, 253 delivery to cells, 254, 256 examples and properties, 254–256 small interfering RNA and gene knockdown experiments glyceraldehyde-3-phosphate dehydrogenase knockdown, 258, 259 light-dosage working curve generation, 258, 261 transfection, 259, 261 transfection, 259,	* * ·	
apoptosis assay, 360–362, 364 proliferation assay, 362 imaging of plates, 356, 357, 359, 360 materials, 353, 354, 363 overview, 353 staining bromodeoxyuridine,	=	
proliferation assay, 362 imaging of plates, 356, 357, 359, 360 materials, 353, 354, 363 overview, 353 staining bromodeoxyuridine, 357–359, 364 Hoechst 33342 counterstaining, 356 YO-PrO-1, 356, 363 transfection, 355, 356 C Caged compounds advantages, 253, 254 batch transfection, 256 cage requirements, 253 delivery to cells, 254, 256 examples and properties, 254–256 small interfering RNA and gene knockdown experiments glyceraldehyde-3-phosphate dehydrogenase knockdown, 258, 259 light-dosage working curve generation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258,		antiproliferative compound assessment in
imaging of plates, 356, 357, 359, 360 materials, 353, 354, 363 overview, 353 staining bromodeoxyuridine, 357–359, 364 Hoechst 33342 counterstaining, 356 YO-PrO-1, 356, 363 transfection, 355, 356 C Caged compounds advantages, 253, 254 batch transfection, 256 cage requirements, 253 delivery to cells, 254, 256 examples and properties, 254–256 small interfering RNA and gene knockdown experiments glyceraldehyde-3-phosphate dehydrogenase knockdown, 258, 259 light-dosage working curve generation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 transfection, 259, 261 rationale, 256 reagent preparation, 130, 131, 137 compound addition, 132 examples, 133, 134, 136, 137 experimental design, 130, 131 image analysis and data visualization, 133 materials, 130 scanning, 132, 133 staining, 132, 133 transfection, 258, 261 cell Lab IC 100, features, 42, 52 Cellome, definition, 4 overview, 5, 6 Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 cellProfiler, image processing and analysis, 37 Cellowics definition, 4 overvie		
materials, 353, 354, 363 overview, 353 staining bromodeoxyuridine,		**
overview, 353 staining bromodeoxyuridine, 357–359, 364 Hoechst 33342 counterstaining, 356 YO-PrO-1, 356, 363 transfection, 355, 356 Caged compounds advantages, 253, 254 batch transfection, 256 cage requirements, 253 delivery to cells, 254, 256 examples and properties, 254–256 small interfering RNA and gene knockdown experiments glyceraldehyde-3-phosphate dehydrogenase knockdown, 258, 259 light-dosage working curve generation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme CellCard preparation, 130, 131, 137 compound addition, 132 examples, 133, 134, 136, 137 experimental design, 130, 131 image analysis and data visualization, 133 materials, 130 scanning, 132, 133 staining, 132, 137 tissue culture, 131 overview of system, 129, 130 Cell counting, fluorescence assays, 240, 241 Cell Lab IC 100, features, 42, 52 Cellome, definition, 4 overview, 5, 6 Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 cellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114– 119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein		
staining bromodeoxyuridine,		
bromodeoxyuridine, 357–359, 364 Hoechst 33342 counterstaining, 356 YO-PrO-1, 356, 363 transfection, 355, 356 C Caged compounds advantages, 253, 254 batch transfection, 256 cage requirements, 253 delivery to cells, 254, 256 examples and properties, 254–256 small interfering RNA and gene knockdown experiments glyceraldehyde-3-phosphate dehydrogenase knockdown, 258, 259 light-dosage working curve generation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme		± ±
357–359, 364 Hocchst 33342 counterstaining, 356 YO-PrO-1, 356, 363 transfection, 355, 356 C C C C C C C C C C C C C C C C C C	_	=
Hoechst 33342 counterstaining, 356 YO-PrO-1, 356, 363 transfection, 355, 356 C Caged compounds advantages, 253, 254 batch transfection, 256 cage requirements, 253 delivery to cells, 254, 256 examples and properties, 254–256 small interfering RNA and gene knockdown experiments glyceraldehyde-3-phosphate dehydrogenase knockdown, 258, 259 light-dosage working curve generation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 transfection, 258, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 smell molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme image analysis and data visualization, 133 materials, 130 scanning, 132, 133 staining, 132, 137 tissue culture, 131 overview of system, 129, 130 Cell counting, fluorescence assays, 240, 241 Cell Lab IC 100, features, 42, 52 Cellomics definition, 4 overview, 5, 6 Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114– 119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein-protein		
YO-PrO-1, 356, 363 transfection, 355, 356 Caged compounds advantages, 253, 254 batch transfection, 256 cage requirements, 253 delivery to cells, 254, 256 examples and properties, 254–256 small interfering RNA and gene knockdown experiments glyceraldehyde-3-phosphate dehydrogenase knockdown, 258, 259 light-dosage working curve generation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme Tagenary 132, 133 statining, 132, 133 statining, 132, 137 tissue culture, 131 overview of system, 129, 130 Cell counting, fluorescence assays, 240, 241 Cellomics definition, 4 overview, 5, 6 Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 Cellomeca assay, 114 112, 118 materials, 130 scanning, 132, 133 cataining, 132, 133 corriew, 132 cell counting, fluorescence assays, 240, 241 Cell bab IC 100, features, 42, 52 Cellomics definition, 4 overview, 5, 6 Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 112, 118 113 Overview of system, 129, 130 Cell counting, fluorescence assays, 240, 241 Cellomics definition, 4 overview, 5, 6 Cellomics automated assay, 111, 112, 118 materials, 109 plating, 111, 117 single cell suspension generation, 111, 111 112, 118 materials, 100 proview of system, 129, 130 Cell counting, fluorescence assays, 240, 241 Cellomics definition, 4 overview, 5, 6 Cellomics definition, 12 112, 11		
transfection, 355, 356 C Caged compounds advantages, 253, 254 batch transfection, 256 cage requirements, 253 delivery to cells, 254, 256 examples and properties, 254–256 small interfering RNA and gene knockdown experiments glyceraldehyde-3-phosphate dehydrogenase knockdown, 258, 259 photoactivation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme materials, 130 scanning, 132, 133 staining, 132, 137 tissue culture, 131 overview of system, 129, 130 Cell counting, fluorescence assays, 240, 241 Cell Lab IC 100, features, 42, 52 Cellome, definition, 3 Cellomics definition, 4 overview, 5, 6 Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 130 scanning, 132, 133 staining, 132, 133 overview of system, 129, 130 Cell counting, fluorescence assays, 240, 241 Cell Lab IC 100, features, 42, 52 Cellomics definition, 4 overview, 5, 6 Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 112, 118 materials, 130 overview of system, 129, 130 Cell counting, fluorescence assays, 240, 241 Cell Lab IC 100, features, 42, 52 Cellome, definition, 3 Cellomics definition, 4 overview, 5, 6 Cell plating, automated materials, 109 plating, 112, 117 single cell suspension generation, 111, 112, 118 materials, 130 overview of system, 129, 130 Cell counting, fluorescence assays, 240, 241 Cell Lab IC 100, features, 42, 52 Cellome, definition, 3 Cellomics definition, 4 overview, 5, 6 Cell plating, 112, 112 117 Single cell suspen		
Caged compounds advantages, 253, 254 batch transfection, 256 cage requirements, 253 delivery to cells, 254, 256 examples and properties, 254–256 small interfering RNA and gene knockdown experiments glyceraldehyde-3-phosphate dehydrogenase knockdown, 258, 259 light-dosage working curve generation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme scanning, 132, 133 tissue culture, 131 overview of system, 129, 130 Cell counting, fluorescene assays, 240, 241 Cell Lab IC 100, features, 42, 52 Cellomics definition, 4 overview, 5, 6 Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemokinesis assay, 111, 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114– 119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein		materials, 130
Caged compounds advantages, 253, 254 batch transfection, 256 cage requirements, 253 delivery to cells, 254, 256 examples and properties, 254–256 small interfering RNA and gene knockdown experiments glyceraldehyde-3-phosphate dehydrogenase knockdown, 258, 259 light-dosage working curve generation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme staining, 132, 137 tissue culture, 129, 130 Cell counting, fluorescene assays, 240, 241 Cell Lab IC 100, features, 42, 52 Cellomics definition, 3 Cellomics definition, 3 Cellomics definition, 4 overview of system, 129, 130 Cell counting, fluorescene assays, 240, 241 Cell Lab IC 100, features, 42, 52 Cellome, definition, 3 Cellomics definition, 3 Cellomics definition, 3 Cellomics definition, 4 overview of system, 129, 130 Cell cab IC 100, features, 42, 52 Cellomics definition, 3 Cellomics definition, 4 overview, 5, 6 Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 Cellomics definition, 3 Cellomics definition	,, ,, ,, ,, ,, ,, ,, ,, ,, ,, ,, ,, ,, ,, ,, ,	
tissue culture, 131 overview of system, 129, 130 Cell counting, fluorescence assays, 240, 241 Cell Lab IC 100, features, 42, 52 Cellome, definition, 3 Cellomics definition, 4 overview, 5, 6 Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114– 119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein—protein	C	
overview of system, 129, 130 Cell counting, fluorescence assays, 240, 241 Cage requirements, 253 delivery to cells, 254, 256 examples and properties, 254–256 small interfering RNA and gene knockdown experiments glyceraldehyde-3-phosphate dehydrogenase knockdown, 258, 259 light-dosage working curve generation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme overview of system, 129, 130 Cell counting, fluorescence assays, 240, 241 Cell Lab IC 100, features, 42, 52 Cellome, definition, 3 Cellomics definition, 4 overview, 5, 6 Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114–119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein	Caged compounds	
batch transfection, 256 cage requirements, 253 delivery to cells, 254, 256 examples and properties, 254–256 small interfering RNA and gene knockdown experiments glyceraldehyde-3-phosphate dehydrogenase knockdown, 258, 259 light-dosage working curve generation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 uCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme Cell counting, fluorescence assays, 240, 241 Cell Lab IC 100, features, 42, 52 Cellome, definition, 3 Cellomics definition, 4 overview, 5, 6 Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemokinesis assay, 111, 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114–119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein		
cage requirements, 253 delivery to cells, 254, 256 examples and properties, 254–256 small interfering RNA and gene knockdown experiments glyceraldehyde-3-phosphate dehydrogenase knockdown, 258, 259 light-dosage working curve generation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme Cell Lab IC 100, features, 42, 52 Cellome, definition, 3 Cellomics definition, 4 overview, 5, 6 Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 Cellomics definition, 4 overview, 5, 6 Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 Cellomics definition, 4 overview, 5, 6 Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 Cellomics definition, 4 overview, 5, 6 Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 Cellomics definition, 4 overview, 5, 6 Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 112, 118 materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 cellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation for displayed assay chemokinesis assay, 111, 112,		
delivery to cells, 254, 256 examples and properties, 254–256 small interfering RNA and gene knockdown experiments glyceraldehyde-3-phosphate dehydrogenase knockdown, 258, 259 light-dosage working curve generation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme Cellome, definition, 3 Cellomics definition, 4 overview, 5, 6 Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114– 119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein	•	
examples and properties, 254–256 small interfering RNA and gene knockdown experiments glyceraldehyde-3-phosphate dehydrogenase knockdown, 258, 259 light-dosage working curve generation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 transfection, 258, 261 transfection, 258, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme Cellomics definition, 4 overview, 5, 6 Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114–119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein		
small interfering RNA and gene knockdown experiments glyceraldehyde-3-phosphate dehydrogenase knockdown, 258, 259 light-dosage working curve generation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme definition, 4 overview, 5, 6 Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 cellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114–119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein		
knockdown experiments glyceraldehyde-3-phosphate dehydrogenase knockdown, 258, 259 light-dosage working curve generation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme overview, 5, 6 Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114– 119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein		definition, 4
glyceraldehyde-3-phosphate dehydrogenase knockdown, 258, 259 light-dosage working curve generation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme Cell plating, automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 Chemotaxis automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 Chemotaxis automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 26 Chemotaxis automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 27 Chemical complementation assay, see Protein phosphatases Chemotaxis automated materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemotary of the protein phosphatases Chemotaxis automated assay chemotary of the protein phosphatases Chemotaxis automated materials, 109 cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation of the protein phosphatases Chemotaxis automated assay chemotary of the protein phosphatases Chemotary of the protein phosphatases Chemotary of the protein phosphatas		•
dehydrogenase knockdown, 258, 259 light-dosage working curve generation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 109 plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 109 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein-protein		
259 light-dosage working curve generation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme plating, 111, 117 single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114– 119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein		
single cell suspension generation, 111, generation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 transfection, 258, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme single cell suspension generation, 111, 117 CellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114– 119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein		
generation, 258, 261 materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme 117 CellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114– 119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein		
materials, 257 photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme CellProfiler, image processing and analysis, 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114– 119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein		
photoactivation, 259–261 rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme 37 CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114– 119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein		CellProfiler, image processing and analysis.
rationale, 256 reagent preparation, 257, 258, 260, 261 transfection, 258, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme CellwoRx, features, 42, 54 Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114– 119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein		
reagent preparation, 257, 258, 260, 261 transfection, 258, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme Chemical complementation assay, see Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114– 119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein	_	CellwoRx, features, 42, 54
261 transfection, 258, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme Protein phosphatases Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114– 119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein		
transfection, 258, 261 transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme Chemotaxis automated assay chemokinesis assay, 111, 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114– 119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein		
transfection, 258, 261 UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme automated assay chemokinesis assay, 111, 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114– 119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein		
UCOM instrumentation for high content screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme chemokinesis assay, 111, 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114– 119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein		automated assay
screening, 254 Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme 112, 118 materials, 110, 111 single cell kinetic and immunocytochemical assay, 114– 119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein		
Calcium flux fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme materials, 110, 111 single cell kinetic and immunocytochemical assay, 114– 119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein		· · · · · · · · · · · · · · · · · · ·
fluorescent probes protein sensors, 236, 237, 235, 236 small molecule probes, 235, 236 mechanisms, 235 CARD, see Catalyzed reporter enzyme single cell kinetic and immunocytochemical assay, 114– 119 directed algorithm for analysis, 76, 77 Coimmunoprecipitation, protein–protein		
protein sensors, 236, 237, immunocytochemical assay, 114– 235, 236 small molecule probes, 235, 236 directed algorithm for analysis, mechanisms, 235 76, 77 CARD, see Catalyzed reporter enzyme Coimmunoprecipitation, protein–protein		
235, 236 small molecule probes, 235, 236 directed algorithm for analysis, mechanisms, 235 76, 77 CARD, see Catalyzed reporter enzyme Coimmunoprecipitation, protein–protein		
small molecule probes, 235, 236 directed algorithm for analysis, mechanisms, 235 76, 77 CARD, see Catalyzed reporter enzyme Coimmunoprecipitation, protein–protein	_	· · · · · · · · · · · · · · · · · · ·
mechanisms, 235 76, 77 CARD, see Catalyzed reporter enzyme Coimmunoprecipitation, protein—protein		
CARD, see Catalyzed reporter enzyme Coimmunoprecipitation, protein–protein		
	,	
	deposition	interactions, 322

Collagen thin film	D
alkanethiol-coated support preparation,	Data mining, see Informatics, high content
102, 103	screening
automated quantitative microscopy of	Data visualization
cells, 99, 104–106	combining data, 307, 308
cells	data sources, 302, 303
culture and specimen preparation, 103	data transformation, 303-307
fixation and staining	filtering, 311
morphology analysis, 104	interactive visualization, 311
green fluorescent protein	overview, 301, 302, 312
quantification, 104, 106	prospects, 311, 312
film preparation and characterization,	related data visualization, 310
103, 105, 106	spatial and temporal views, 309
materials for quantitative measurements	tools, 275, 276
of cells,	well- and plate-level information, 309,
101, 102	310
reference extracellular matrix	Differentiating cell systems
application, 96–99	adipocytes
variability within cell populations, 99,	preadipocyte culture, 122, 123, 126,
100	127
Computer vision	triglyceride accumulation assay, 123,
image analysis workflow, 84, 85	124, 127
limitations of conventional imaging	applications, 121, 122
systems, 85–87	materials, 122, 125, 126
Cytotoxicity	osteoclasts
assay limitations and effective criteria,	precursor culture, 124, 127
419–421	resorption assay, 125, 127
cellular functions in assessment,	Directed algorithms
421, 422	associated target identification and
drug discovery	measurement, 70, 71
assay design, 423	automated directed algorithm analysis
implementation of cytotoxicity	steps, 66
assessment, 422, 423	BioApplications, 64
importance of cytotoxicity assessment,	developer tools versus specific
415, 416	algorithms, 65, 66
genotoxicity, high content screening	high content screening problems
assays, 381, 384–386	categorization of problems
hepatotoxicity quad probe assay, 424	cell cycle, 78
mechanisms	cell health and toxicity, 79
calcium dyshomeostasis, 419	cell movement, 76, 77
cell membrane effects and transport, 416	cell size and shape changes, 75, 76
lysosomal effects and autophagy, 417	colocalization, 74, 75
mitochondrial effects and energy	counting objects, 73, 74
homeostasis, 416, 417	interconnected tubular object
nuclear effects and cell proliferation, 416	analysis, 76
oxidative stress, 416–418, 419	intracellular intensity changes,
necrosis	72, 73
conventional assays, 423	neurite outgrowth, 78
high content screening, 423, 424	overview, 72, 73

receptor internalization and	immunofluorescence of small interfering
translocation, 77, 78	RNA treated cells, 249–251
spot analysis, 74	lifetime imaging, 59
translocation, 77	light sources, 45, 46
practical utilization of directed	multiwavelength imaging, 45, 59
algorithms, 79, 80	numerical aperture of objectives,
raw measurement analysis, 71, 72	47, 48
primary objects	optical performance parameters,
identification, 68	46, 47
property measurements, 68	standards, 241
Discovery-1, features, 42, 54, 55	Fluorescence resonance energy transfer
Dual-specificity phosphatases, see Protein	(FRET), green fluorescent protein
phosphatases	applications, 150
n n	Fluorescent probes, see also specific probes
E–F	brightness, 144
Extracellular matrix protein, see Collagen	cellular manipulation combination with
thin film	high content screening, 155
FlAsH	cellular probes, 238–241
applications, 210, 211, 218, 219	classification, 143, 144
protein conformation studies,	collection modalities and options, 234,
210, 218	235
protein labeling in live cells	fixed end-point high content screening
background staining reduction/	reagents
suppression, 216–218	aptamers, 147
expression, 214, 215, 219	catalyzed reporter enzyme deposition
FlAsH loading and staining, 215, 216,	147
219	immunoreagents, 145, 146
fluorescence microscopy,	molecular beacons, 147
218, 219	quantum dots, 146, 147
materials, 211, 212	four-color multiplexed immunoassay
tetracysteine-tagged protein	design
generation, 212, 213, 218	cell plating and incubation,
protein tagging, 180, 181	190, 191
tetracysteine binding and fluorescence	materials, 190, 191
induction, 210	overview, 189, 190 plate reading and interpretation, 191
Flash photolysis, see Caged compounds	staining, 190–192
Flow cytometry	ion indicators, 235–237, 239
historical perspective, 6–8	live cell and kinetic high content
standards, 241 Fluorescence microscopy	screening reagents
autofocus and system performance, 48,	fluorescent analog cytochemistry
49	applications, 149
automation, 11, 64	cell loading, 149, 150
cameras, 49	green fluorescent protein fusion
confocal versus wide-field systems, 53,	proteins, 150
54	living cell probe capture, 150
FlAsH in living cells, 218, 219	overview, 147, 148
historical perspective, 6–11	fluorescent protein biosensors
r, 0 22	engineering, 152, 153

	cell engineering for fusion protein
transfer, 153, 154	expression
pH indicators, 154	cell line selection, 171
prospects, 154	cell selection for expression, 177, 178
translocation studies, 154	design elements, 168, 170
physiological indicator dyes,	fluorescent protein variant selection,
147, 148	172
positional biosensors, 150–152	stable transfectant validation, 178
live cell experiment design, 234, 235	target protein selection, 168,
nonspecific binding, 144	170, 171
perturbing reactions, 145	vector design, 172, 174, 176
photobleaching, 144	collagen thin film cell fixation and
phototoxicity, 144	staining for automated microscopy,
prospects, 155, 156	104, 106
stability, 144	expression effects on cell cycle, 178-180
standards, 241, 242	fluorescent analog cytochemistry, 150
vendors, 233, 234	high content screening application
voltage-sensing dyes, 237	overview, 167, 168
Forkhead assay, principles, 402,	history of research use, 65
404, 411	limitations, 209
FRET, see Fluorescence resonance energy	prospects, 180, 181
transfer	variants and properties, 165, 166
G	Н
GAPDH, see Glyceraldehyde-3-phosphate	HaloTag TM
dehydrogenase	advantages, 204, 205
Gene MicroArray Pathway Profiler,	cell culture, transfection, and labeling
pathway database, 321	with ligands, 201, 205
	with figures, 201, 203
Genotoxicity, see Cytotoxicity	cell-based applications, 197
Genotoxicity, see Cytotoxicity GFP, see Green fluorescent protein	
	cell-based applications, 197
GFP, see Green fluorescent protein	cell-based applications, 197 expression vectors, 198–201
GFP, <i>see</i> Green fluorescent protein Glyceraldehyde-3-phosphate dehydrogenase	cell-based applications, 197 expression vectors, 198–201 fixation and immunocytochemistry, 201
GFP, <i>see</i> Green fluorescent protein Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), caged small interfering	cell-based applications, 197 expression vectors, 198–201 fixation and immunocytochemistry, 201 fluorescence detection, 203, 204
GFP, see Green fluorescent protein Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), caged small interfering RNA knockdown, 258, 259 GPCRs, see G protein-coupled receptors	cell-based applications, 197 expression vectors, 198–201 fixation and immunocytochemistry, 201 fluorescence detection, 203, 204 hydrolase reporter, 195, 196,
GFP, see Green fluorescent protein Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), caged small interfering RNA knockdown, 258, 259	cell-based applications, 197 expression vectors, 198–201 fixation and immunocytochemistry, 201 fluorescence detection, 203, 204 hydrolase reporter, 195, 196, 205, 206
GFP, see Green fluorescent protein Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), caged small interfering RNA knockdown, 258, 259 GPCRs, see G protein-coupled receptors G protein-coupled receptors (GPCRs)	cell-based applications, 197 expression vectors, 198–201 fixation and immunocytochemistry, 201 fluorescence detection, 203, 204 hydrolase reporter, 195, 196, 205, 206 ligand structures, 195, 196
GFP, see Green fluorescent protein Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), caged small interfering RNA knockdown, 258, 259 GPCRs, see G protein-coupled receptors G protein-coupled receptors (GPCRs) activation, 20 high content screening of orphan	cell-based applications, 197 expression vectors, 198–201 fixation and immunocytochemistry, 201 fluorescence detection, 203, 204 hydrolase reporter, 195, 196,
GFP, see Green fluorescent protein Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), caged small interfering RNA knockdown, 258, 259 GPCRs, see G protein-coupled receptors G protein-coupled receptors (GPCRs) activation, 20 high content screening of orphan receptors, 21, 22, 30	cell-based applications, 197 expression vectors, 198–201 fixation and immunocytochemistry, 201 fluorescence detection, 203, 204 hydrolase reporter, 195, 196,
GFP, see Green fluorescent protein Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), caged small interfering RNA knockdown, 258, 259 GPCRs, see G protein-coupled receptors G protein-coupled receptors (GPCRs) activation, 20 high content screening of orphan receptors, 21, 22, 30 protein-fragment complementation	cell-based applications, 197 expression vectors, 198–201 fixation and immunocytochemistry, 201 fluorescence detection, 203, 204 hydrolase reporter, 195, 196,
GFP, see Green fluorescent protein Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), caged small interfering RNA knockdown, 258, 259 GPCRs, see G protein-coupled receptors G protein-coupled receptors (GPCRs) activation, 20 high content screening of orphan receptors, 21, 22, 30	cell-based applications, 197 expression vectors, 198–201 fixation and immunocytochemistry, 201 fluorescence detection, 203, 204 hydrolase reporter, 195, 196,
GFP, see Green fluorescent protein Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), caged small interfering RNA knockdown, 258, 259 GPCRs, see G protein-coupled receptors G protein-coupled receptors (GPCRs) activation, 20 high content screening of orphan receptors, 21, 22, 30 protein-fragment complementation assays for dynamics studies, 228–	cell-based applications, 197 expression vectors, 198–201 fixation and immunocytochemistry, 201 fluorescence detection, 203, 204 hydrolase reporter, 195, 196,
GFP, see Green fluorescent protein Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), caged small interfering RNA knockdown, 258, 259 GPCRs, see G protein-coupled receptors G protein-coupled receptors (GPCRs) activation, 20 high content screening of orphan receptors, 21, 22, 30 protein-fragment complementation assays for dynamics studies, 228– 230	cell-based applications, 197 expression vectors, 198–201 fixation and immunocytochemistry, 201 fluorescence detection, 203, 204 hydrolase reporter, 195, 196,
GFP, see Green fluorescent protein Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), caged small interfering RNA knockdown, 258, 259 GPCRs, see G protein-coupled receptors G protein-coupled receptors (GPCRs) activation, 20 high content screening of orphan receptors, 21, 22, 30 protein-fragment complementation assays for dynamics studies, 228– 230 Green fluorescent protein (GFP) biosensors	cell-based applications, 197 expression vectors, 198–201 fixation and immunocytochemistry, 201 fluorescence detection, 203, 204 hydrolase reporter, 195, 196,
GFP, see Green fluorescent protein Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), caged small interfering RNA knockdown, 258, 259 GPCRs, see G protein-coupled receptors G protein-coupled receptors (GPCRs) activation, 20 high content screening of orphan receptors, 21, 22, 30 protein-fragment complementation assays for dynamics studies, 228– 230 Green fluorescent protein (GFP) biosensors engineering, 152, 153	cell-based applications, 197 expression vectors, 198–201 fixation and immunocytochemistry, 201 fluorescence detection, 203, 204 hydrolase reporter, 195, 196,
GFP, see Green fluorescent protein Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), caged small interfering RNA knockdown, 258, 259 GPCRs, see G protein-coupled receptors G protein-coupled receptors (GPCRs) activation, 20 high content screening of orphan receptors, 21, 22, 30 protein-fragment complementation assays for dynamics studies, 228– 230 Green fluorescent protein (GFP) biosensors engineering, 152, 153 fluorescence resonance energy	cell-based applications, 197 expression vectors, 198–201 fixation and immunocytochemistry, 201 fluorescence detection, 203, 204 hydrolase reporter, 195, 196,
GFP, see Green fluorescent protein Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), caged small interfering RNA knockdown, 258, 259 GPCRs, see G protein-coupled receptors G protein-coupled receptors (GPCRs) activation, 20 high content screening of orphan receptors, 21, 22, 30 protein-fragment complementation assays for dynamics studies, 228– 230 Green fluorescent protein (GFP) biosensors engineering, 152, 153 fluorescence resonance energy transfer, 153, 154	cell-based applications, 197 expression vectors, 198–201 fixation and immunocytochemistry, 201 fluorescence detection, 203, 204 hydrolase reporter, 195, 196,
GFP, see Green fluorescent protein Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), caged small interfering RNA knockdown, 258, 259 GPCRs, see G protein-coupled receptors G protein-coupled receptors (GPCRs) activation, 20 high content screening of orphan receptors, 21, 22, 30 protein-fragment complementation assays for dynamics studies, 228– 230 Green fluorescent protein (GFP) biosensors engineering, 152, 153 fluorescence resonance energy	cell-based applications, 197 expression vectors, 198–201 fixation and immunocytochemistry, 201 fluorescence detection, 203, 204 hydrolase reporter, 195, 196,

informatics, see Informatics, high content	implementation decisions, 285
screening	information explosion, 293, 294
installation and operation considerations,	information retrieval challenges, 294
56, 57	metadata structure, 272
market and commercial suppliers, 43	organizational structure
overview, 4, 5, 379	championing research needs, 283
prospects, 14–16, 31	core activities, 283, 284
spatial resolution, 34, 44	noncore activities, 284
throughput, 43, 56	partnering, 282
time-resolved assays, 35, 36	results of research organization/
whole-tissue assays, 36	information technology partnering,
HPRD, see Human Protein Reference	284
Database	trust building, 283
Human Protein Reference Database	overview, 269, 270
(HPRD), protein-protein	prospects, 279, 291
interactions, 323	research needs for computing,
_	281, 282
I	system architecture, 272, 273
Image analysis, see also Computer vision;	Velocity for Life Sciences TM , see
Machine learning; Software	Velocity for Life Sciences TM
data mining, 52, 53	visualization, see Data visualization
data storage and management, 53	volume of data, 270, 271
ImageExpress 5000A, features, 42	iPath, pathway database, 322
ImageExpress Micro, features, 42,	
49, 54	J–M
Imaging, see Fluorescence microscopy;	JNK, see Jun N-terminal kinase
Image analysis; Software	Jun N-terminal kinase (JNK), inhibitor
ImageJ, image processing and	discovery with high content
analysis, 37	screening, 380–382
ImagePro, image analysis, 50, 64	KEGG, pathway database, 321
InCell 1000, features, 42, 54, 55	KineticScan ^(r) , features, 42
InCell 3000, features, 42, 45, 55, 56	Kolmogorov-Smimov statistics, high
Informatics, high content screening	content single-cell chemical
data characteristics, 270, 271,	complementation assay analysis,
279, 280	395
data integration with other systems	Lead candidates, high content screening, 22,
application/software integration, 278,	24–26
279	Machine learning
database integration/federation, 289	custom solutions, 92-94
data-level integration, 278	imaging systems
overview, 276	classification, 87–89
data management, 275	image analysis workflow, 87
data mining, 276	segmentation, 89–91
database structure, 272	limitations of conventional imaging
functional workflow, 294, 295	systems, 85–87
hardware and network considerations,	overview, 85
273–275	training techniques, 91–93
high throughput screening,	MetaCore
285–289, 291	architecture

block, 327, 328	Necrosis, see Cytotoxicity
component, 327	Networks
effect, 327	definition, 320
overview, 326	information flow in cells, 320, 321
transformation, 327	MetaCore, see MetaCore
content, 326	theory and tools for analysis,
experimental data mapping on networks,	323–326
330	Neural networks, see Machine learning
genomics data mapping	Neurite outgrowth
signature networks for radiosensitive	activator discovery with high content
cervical cancer patients, 340	screening, 381, 384, 386
Tat-upregulated genes at G1/S phase,	automated assay
336, 339	addition of analytes, 112, 118
high content screening data mapping on	fixation and labeling, 113,
networks, 336	114, 118
metabonomics data mapping, 336	imaging of plates, 114
network algorithm and filters	materials, 110
analyze networks algorithm, 329	directed algorithm for analysis, 78
analyze transcriptional regulation	retinal ganglion cell growth promoter
algorithm, 329	screening
auto expand algorithm, 329	cell purification and culture, 431
direct interactions algorithm, 329	image analysis, 432
expand by one interaction algorithm,	materials, 430
330	principles, 429–431
overview, 328, 329	staining and imaging, 431, 432
self regulations algorithm, 329	statistical analysis, 432
shortest paths algorithm, 329	validation of hits, 432, 433
network statistical analysis, 330, 332	NLP, see Natural language processing
overview, 345, 346	N-tier architecture, informatics
pathway database, 321, 322	systems, 273
proteomics data mapping,	Nuclear translocation, see Protein
340–342, 345	translocation
<i>p</i> -value and statistical significance	О-Р
evaluation, 332, 333	
MetaDrug	Opera, features, 42, 45, 54, 56
applications, 334–336	Organelles, fluorescent probes, 239
overview of features, 334, 345, 346	Osteoclast, see Differentiating cell systems
prospects, 334	p53, Hdm2 interaction assay, 404
MetaMorph, image analysis, 50	PathArt, features, 334
Mitogen-activated kinase phosphatases, see	Pathway
Protein phosphatases Melagular bassans, fixed and point high	databases, 320–323
Molecular beacons, fixed end-point high	definition, 320
content screening assays, 147 Morphology profiling, subpopulation	high content translocation assays, <i>see</i> Protein translocation
dynamics, 35	
dynamics, 33	information flow in cells, 320, 321
N	profiling in drug discovery, 402
	Pathway Analysis, features, 333
Natural language processing (NLP), data mining, 322	Pathway HT, features, 42, 54–56 Pathway Studio, features, 334
11111111111111111111111111111111111111	1 aniway Studio, icatules, 334

PCA, see Protein-fragment	transcription factor activation, 382,
complementation assay	383, 385, 386
Phosphatases, see Protein phosphatases	pathway profiling
Phosphatidylinositol-3-kinase (PI3K),	Akt pathway, 411
translocation analysis of pathway,	cell lines, 404, 405, 411, 412
411	clone testing, 408
PI3K, see Phosphatidylinositol-3-kinase	expression vectors, 405, 406
PKA, see Protein kinase A	fluorescent protein selection and
PKC, see Protein kinase C	orientation, 405, 406
Plating, see Cell plating, automated	optimization of assay, 408, 409
Potassium, fluorescent probes, 239	overview, 401, 402
Protein kinase A (PKA), fluorescent cyclic	pathway function assessment, 410-
AMP biosensors,	413
150–152	phosphatidylinositol-3-kinase
Protein kinase C (PKC), translocation	pathway, 411
inhibitor discovery with high	target selection, 405
content screening, 380, 383, 384,	target validation, 409, 410
386	transfectant selection and testing,
Protein Lounge, pathway database, 321	407, 408
Protein phosphatases	transfection, 406, 407
dual-specificity phosphatases, 389	plasma membrane translocation
high content single-cell chemical	GLUT4 assay, 404
complementation assay	materials, 380
cell transfection, treatment, and	p53-Hdm2 assay, 404
processing, 392–394, 399 data set evaluation	protein kinase C/Akt assay, 383–386
archived image inspection, 396,	Protein-fragment complementation assay
397	(PCA)
false-positives, 395, 396	drug discovery advantages, 226
Kolmogorov–Smimov statistics,	drug effect studies, 230
395	pharmacological profiling, 230, 231
software, 394, 395, 399	principles, 223–225
subpopulation analysis,	protein complexes
395, 399	drug interactions, 221–223
image acquisition and	dynamics studies, 228–230
analysis, 394	localization and quantification in
materials, 391, 392	cells, 223–225
principles, 390	subcellular localization of protein
prospects, 398, 399	complexes, 226, 228
secondary assays, 397, 398	<i>p</i> -value, network statistical significance
mitogen-activated kinase phosphatases,	evaluation, 332, 333
389	, ,
Protein translocation	Q-R
directed algorithms, 77	Quantum dots, fixed end-point high content
fluorescent proteins, 154, 401, 402	screening assays,
high content screening	146, 147
nuclear translocation	Raf, protein-fragment complementation
Forkhead assay, 402, 404, 411	assays for dynamics studies, 229,
materials, 380	230

Ras, protein-fragment complementation	cell plating for transfection,
assays for dynamics studies, 229,	246, 247
230	immunofluorescence of small
ReAsH, principles, 210	interfering RNA treated cells, 249-
Reference standards, high content	251
screening, 57, 58	materials, 246, 251 overview, 245, 246
Retinal ganglion cell (RGC) apoptosis following optic nerve injury,	siIMPORTERTM transfection
427, 428	reagent, 247, 251
axonal growth factors and identification,	small interfering RNA expression
428, 429, 433	plasmids
neurite outgrowth promoter screening	oligonucleotide design and cloning
cell purification and culture, 431	248
image analysis, 432	transfection, 248
materials, 430	transfection, 248 transfection complex, 248, 251
principles, 429–431	transfection with small interfering
staining and imaging, 431, 432	RNA duplexes, 247, 248
statistical analysis, 432	physiological functions, 245
validation of hits, 432, 433	sample manipulation, 36
pathology, 427	target validation, 409, 410
RGC, see Retinal ganglion cell	siRNA, see Small interfering RNA
RNA interference, see also Small	Small interfering RNA (siRNA), see also
interfering RNA	RNA interference
genomic screens for gene target	caged small interfering RNA and gene
identification, 245	knockdown experiments
high content screening cancer studies of	glyceraldehyde-3-phosphate
gene knockdown effects on	dehydrogenase knockdown, 258,
apoptosis and proliferation	259
cells	light-dosage working curve
seeding on plates, 355, 363	generation, 258, 261
selection of cell lines, 354, 363	materials, 257
data analysis	photoactivation, 259–261
apoptosis analysis, 362	rationale, 256
proliferation analysis, 362, 363	reagent preparation, 257, 258, 260,
image processing	261
apoptosis assay, 360–362, 364	transfection, 258, 261
proliferation assay, 362	transfection, 258, 261
imaging of plates, 356, 357,	profiling, 28
359, 360	SNAP-tag, protein tagging, 181
materials, 353, 354, 363	Sodium, fluorescent probes, 239
overview, 353	Software
staining	commercial sources by application, 50-
bromodeoxyuridine,	52
357–359, 364	customization, 28–30, 92–94
Hoechst 33342 counterstaining,	directed algorithms, see Directed
356	algorithms
YO-PrO-1, 356, 363	imaging, see also Computer vision
transfection, 355, 356	acquisition and control, 50
immunofluorescence study validation	analysis, 37, 50–52

integration between systems, 278, 279	Tetracysteine, see FlAsH Transcription factors, high content
machine learning, <i>see</i> Machine learning prospects for automated assay	screening, 26, 402, 404, 411
development, 59	V-Y
statistical analysis, 394, 399	Velocity for Life Sciences TM (VVLS)
Systems cell biology	advantages, 300
definition, 4	content integration, 296, 297
levels, 5	document clustering, 296
	intelligent query routing, 296–300
T	
Target validation	Visualization, see Data visualization
Akt signaling pathway high content screening assays	Voltage-sensing dyes, fluorescent probes, 236, 237
cell line selection, 368, 373	VVLS, see Velocity for Life Sciences TM
immunoassays of phosphorylated	Western blot
proteins, 372, 373–375	HaloTag protein fusions, 204
materials, 368	target validation, 409, 410
multiplexed apoptosis and	Yeast, high content screening applications,
proliferation assay, 373–375	33, 34
reagent identification, 368–370, 373,	Yeast two-hybrid system, protein-protein
374	interactions, 322
directed algorithms, 70, 71	
overview of process, 367	