

Scan Times: Standard Scans

(96-well Plate; Aq time = 400 ms Green, 800 ms Red)

Objective	Channel(s)	1 per well	2 per well	3 per well	4 per well	9 per well
4x	Phase	3	-	-	-	-
	Phase + Green	4	-	-	-	-
	Phase + Red	5	-	-	-	-
	Phase + Green + Red	6	-	-	-	-
10x	Phase	3	6	8	11	-
	Phase + Green	4	8	12	15	-
	Phase + Red	5	9	13	18	-
	Phase + Green + Red	6	11	16	21	-
20x	Phase	4	7	11	14	28
	Phase + Green	5	10	14	19	38
	Phase + Red	6	11	16	21	44
	Phase + Green + Red	7	12	18	24	50

Time in minutes to complete scan of a 96-well plate (Estimated)

Image Statistics: Standard Scans

Objective	Sampling Area	Resolution	96 well coverage/image	Image size (pixels)
4x	4.25 mm x 3.17 mm; 13.46 mm ²	3.05 µm/pixel	42.06%	1382 x 1040
10x	1.70 mm x 1.27 mm; 2.15 mm ²	1.22 µm/pixel	6.72%	1382 x 1040
20x	0.85 mm x 0.63 mm; 0.54 mm ²	0.61 µm/pixel	1.69%	1382 x 1040

- When possible, use the 4x objective for significant time savings and greater sampling
 - 4x has been tested and has worked well for most CellPlayer applications (≠ NeuroTrack)

Example: 4x, 3 channels, 1 image per well, 42% well sampled = 6 minutes / plate

10x, 3 channels, 4 images per well, 26.8% well sampled = 21 minutes / plate

- Essen Recommends:

- For shorter scan times and better sampling: 4x, 1 image per well
- For more resolution and sufficient sampling: 10x, 2 images per well

Scan Times and Image Statistics: 384-well Plates

Objective	Phase	Phase + Green	Phase + Red	Phase + Green + Red
10x	11	16	18	21
20x	15	19	22	25

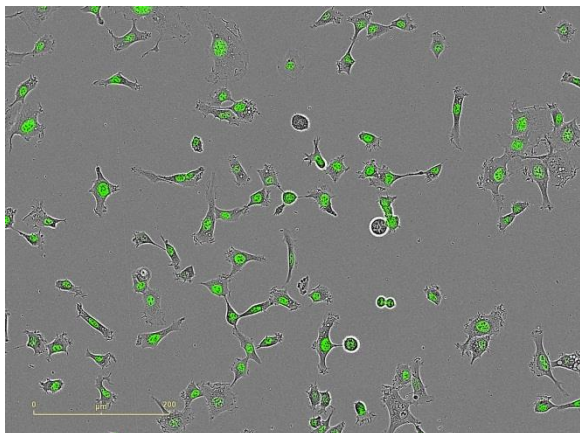
Objective	Sampling Area	Resolution	96 well coverage/image	Image size (pixels)
10x	1.70 mm x 1.27 mm; 2.15 mm ²	1.22 µm/pixel	35.8%	1382 x 1040
20x	0.85 mm x 0.63 mm; 0.54 mm ²	0.61 µm/pixel	9%	1382 x 1040

- Time in minutes to complete scan of a 384-well plate (Estimated)
- Can only scan 1 image per well for 10x and 20x when using a 384-well plate
- Unable to image 384-well plates using a 4x objective
- Acquisition Time = 400 ms for Green channel, 800 ms for Red channel

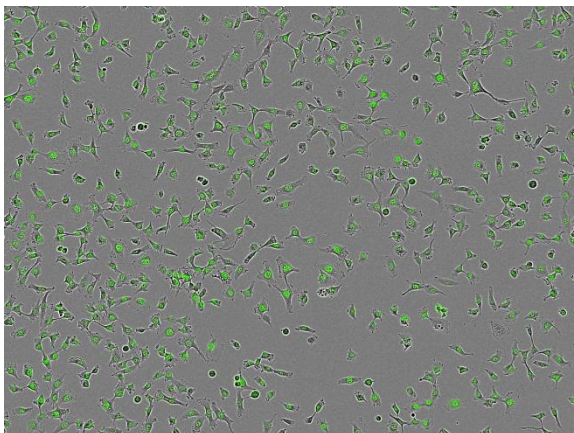
HT-1080 NucLight Green at Different Magnifications

Native Image Size: 1392 x 1040 pixels

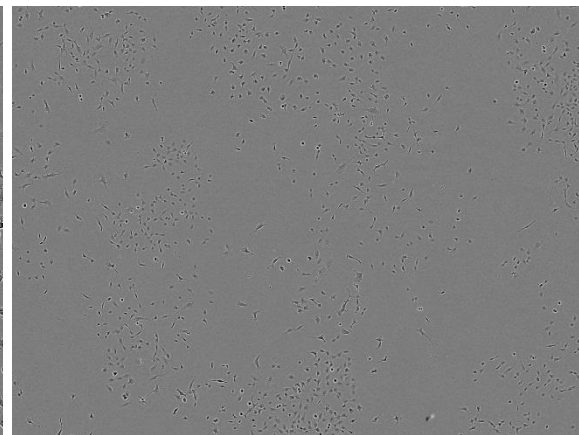
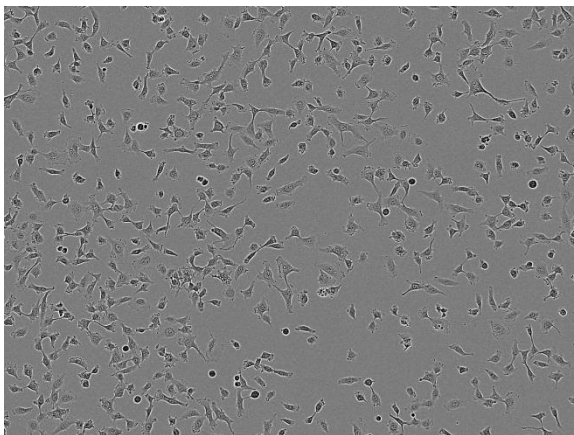
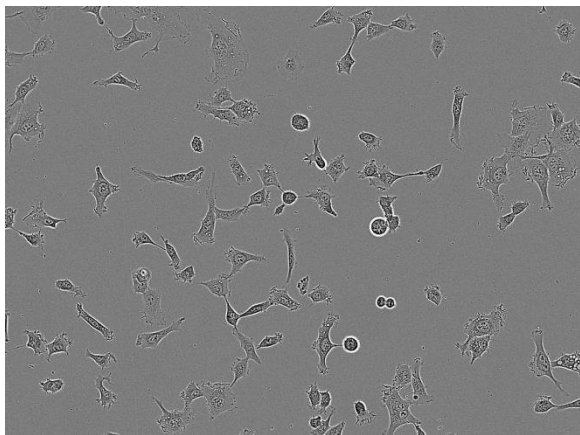
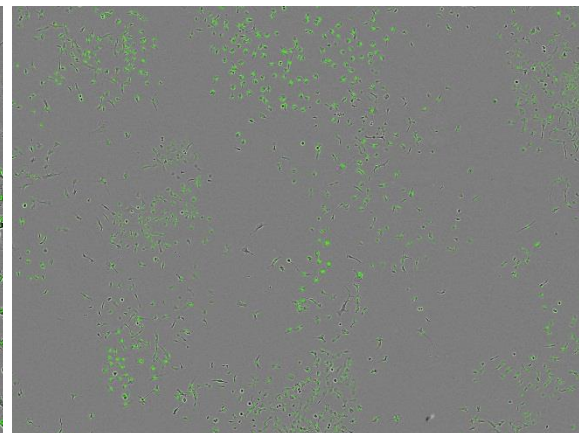
20x – 0.61 $\mu\text{m}/\text{pixel}$



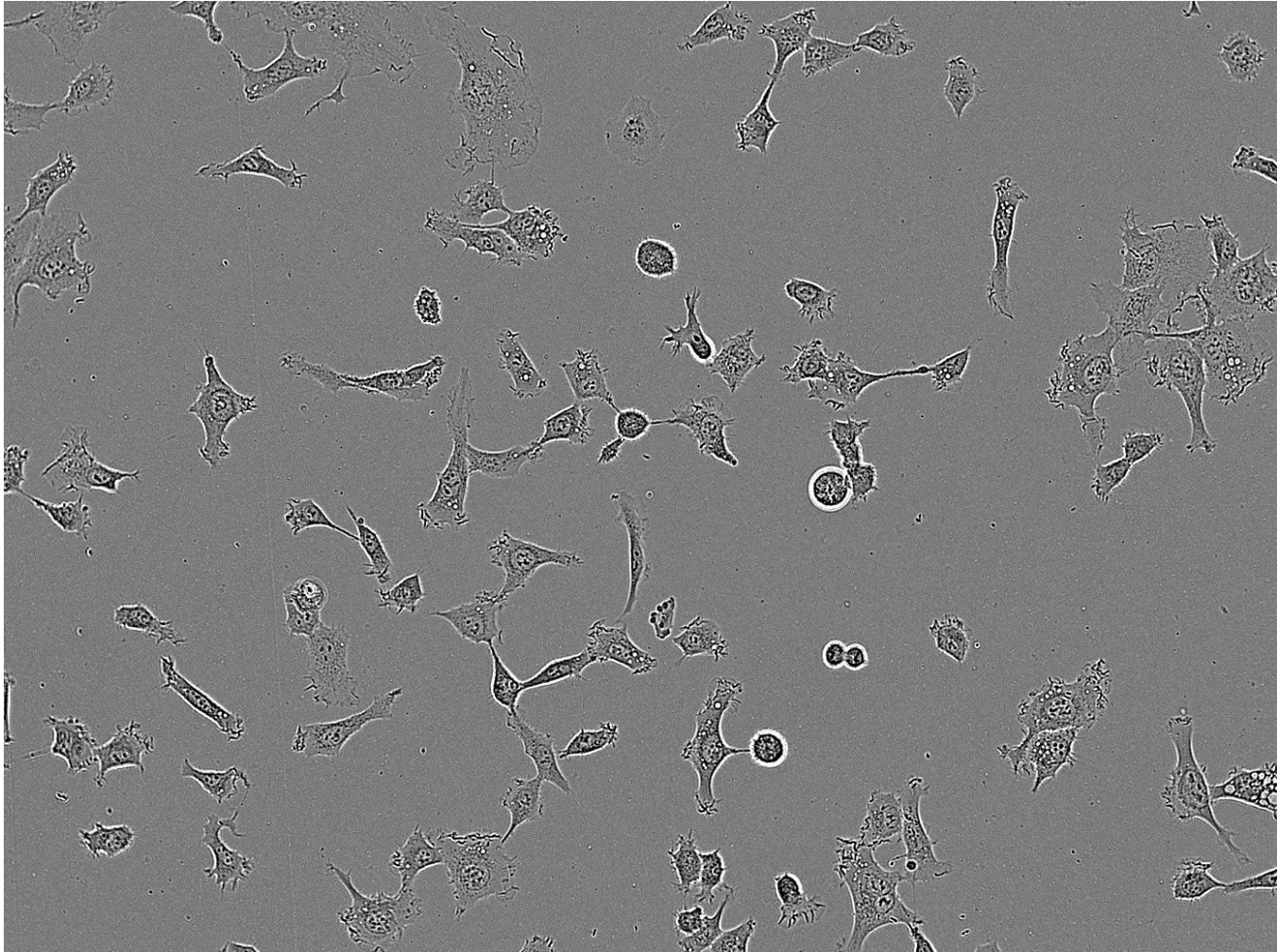
10x – 1.22 $\mu\text{m}/\text{pixel}$



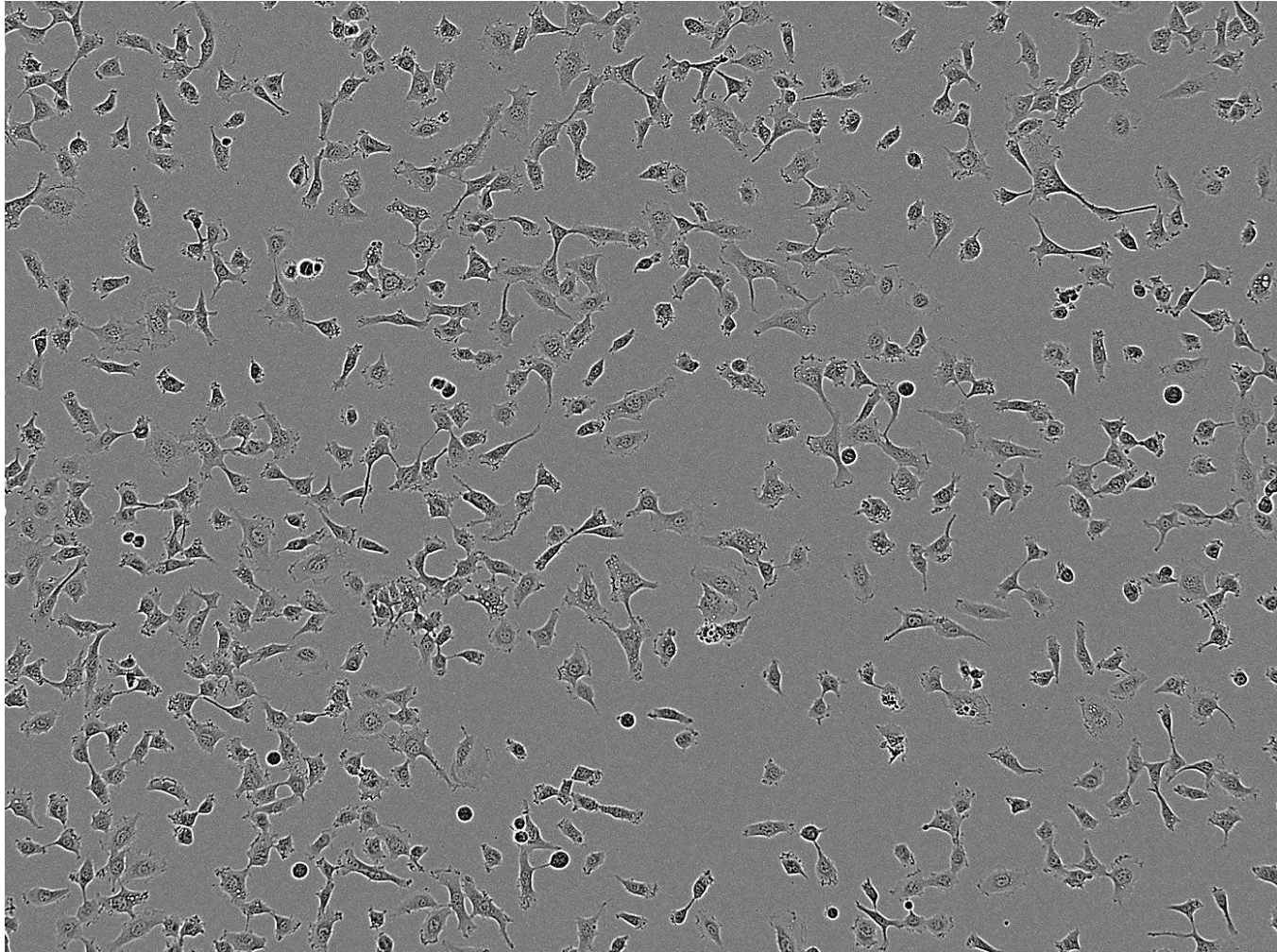
4x – 3.05 $\mu\text{m}/\text{pixel}$



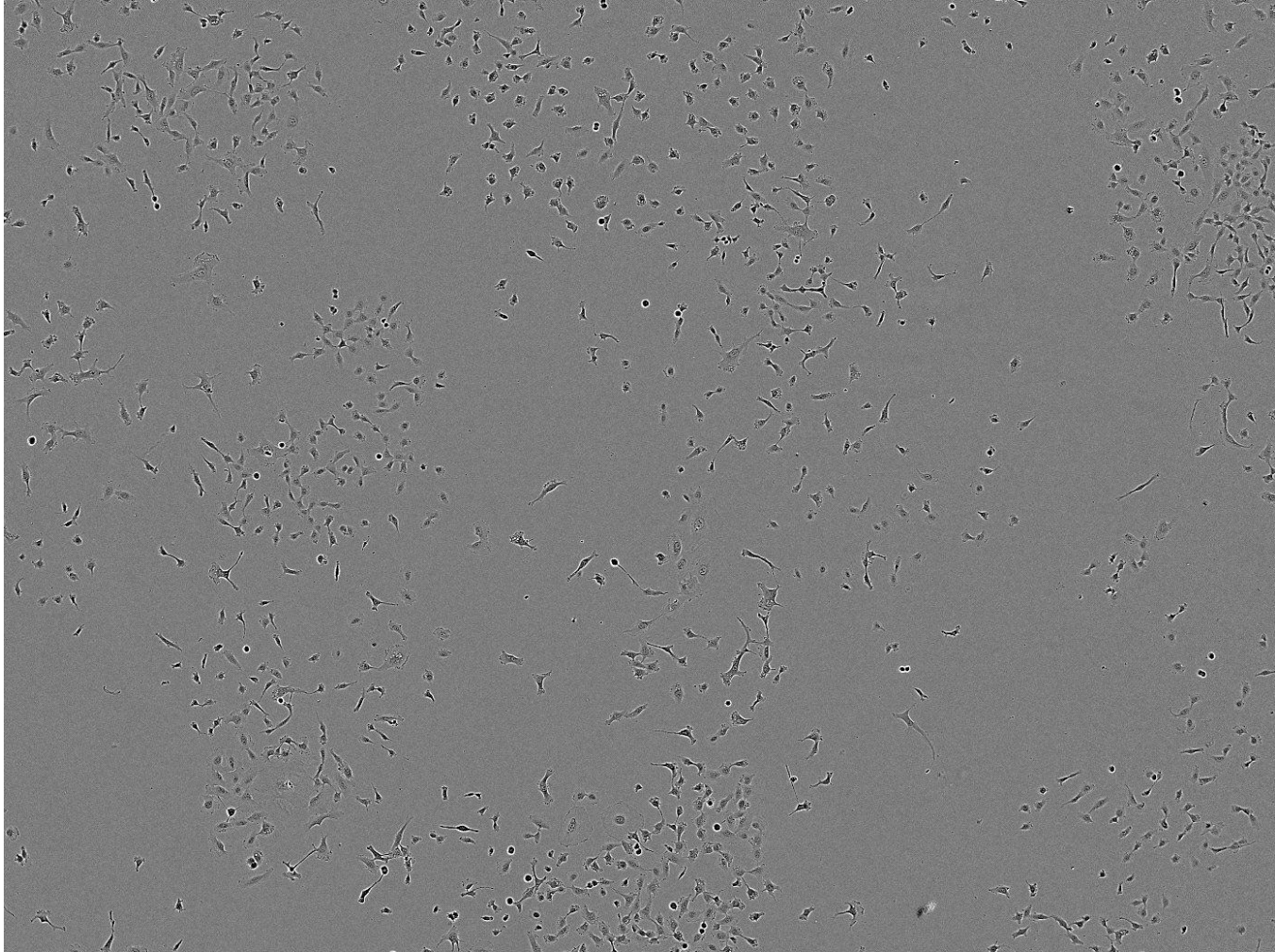
20x – 0.61 $\mu\text{m}/\text{pixel}$



10x – 1.22 $\mu\text{m}/\text{pixel}$



4x – 3.05 $\mu\text{m}/\text{pixel}$



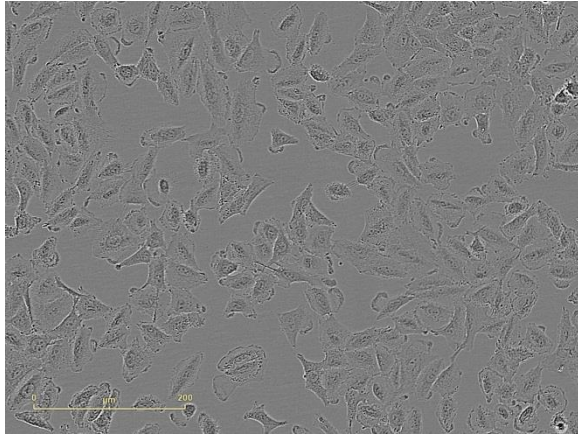
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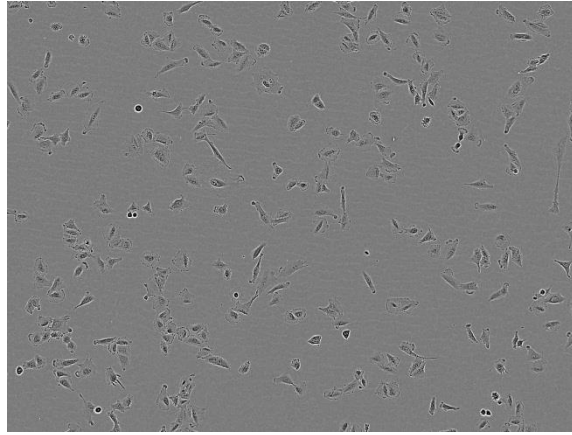
A549 NucLight Red at Different Magnifications

Native Image Size: 1392 x 1040 pixels

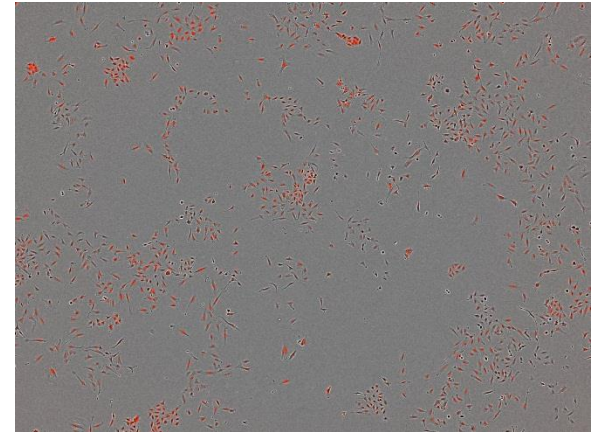
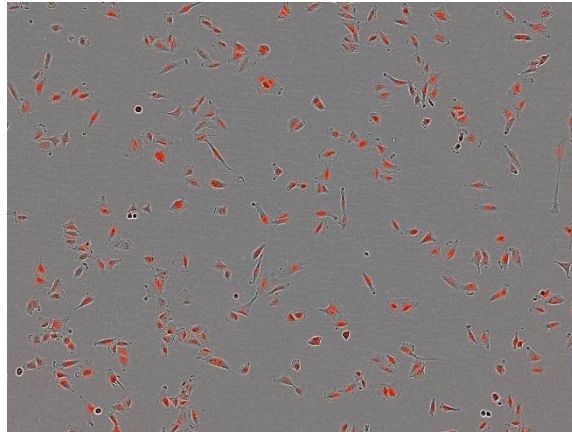
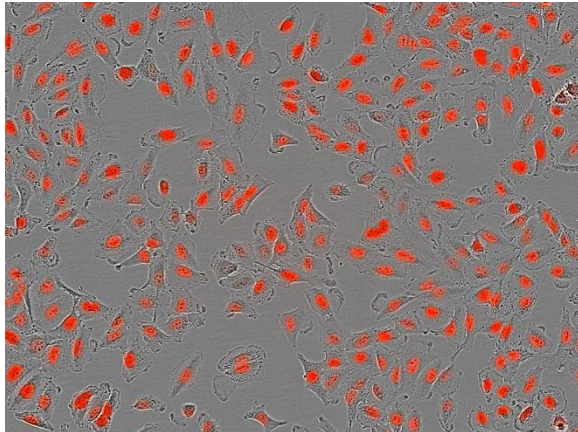
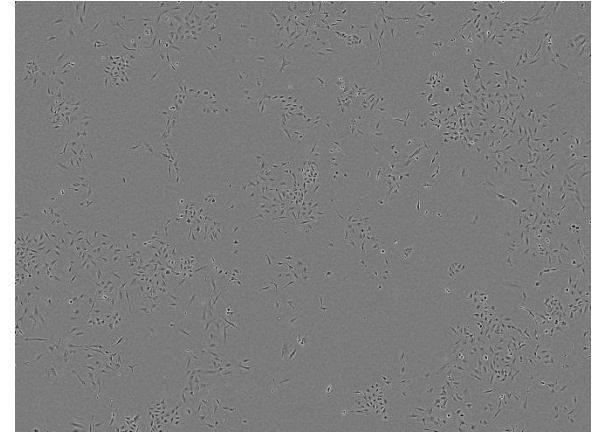
20x – 0.61 $\mu\text{m}/\text{pixel}$



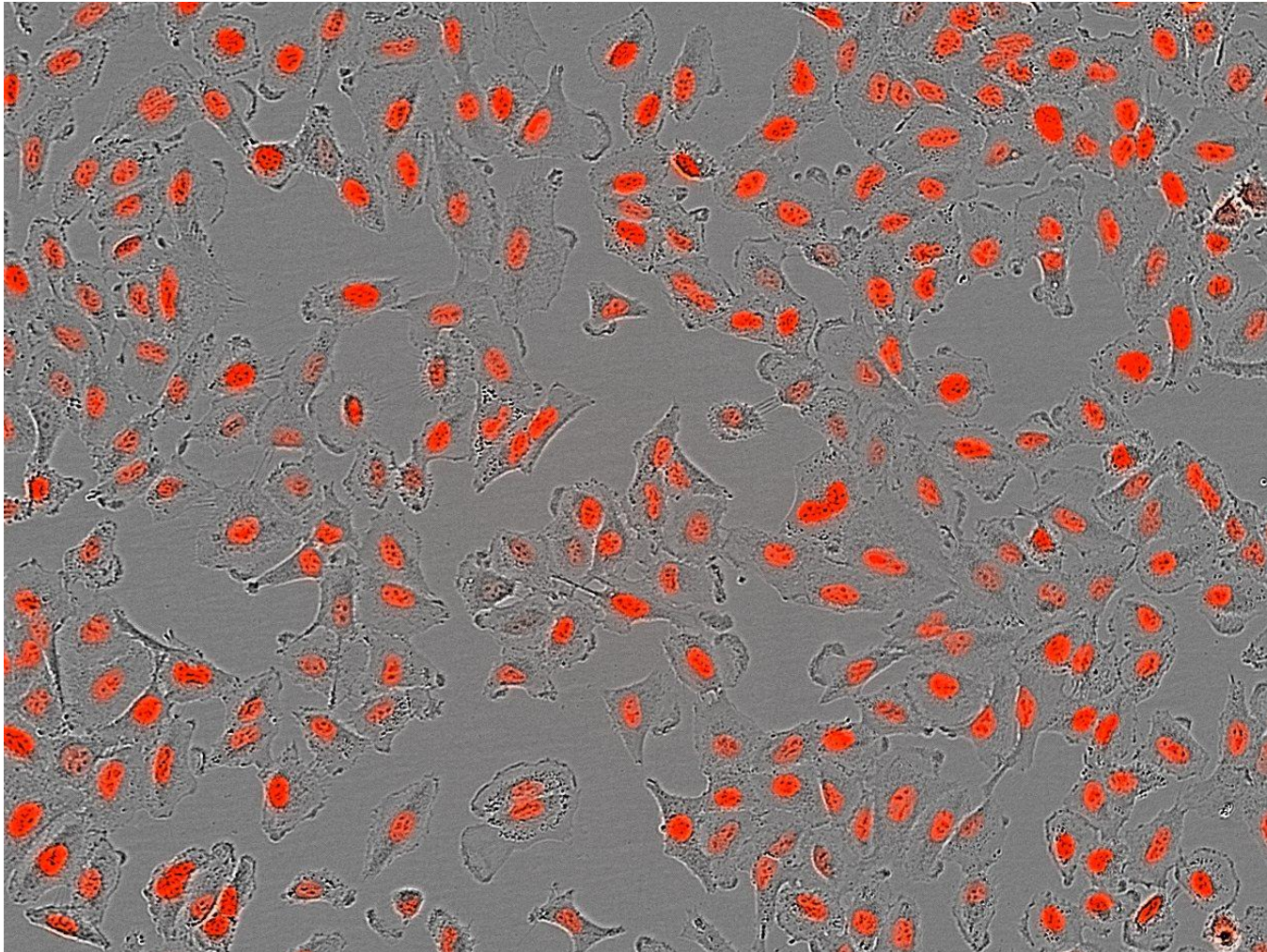
10x – 1.22 $\mu\text{m}/\text{pixel}$



4x – 3.05 $\mu\text{m}/\text{pixel}$



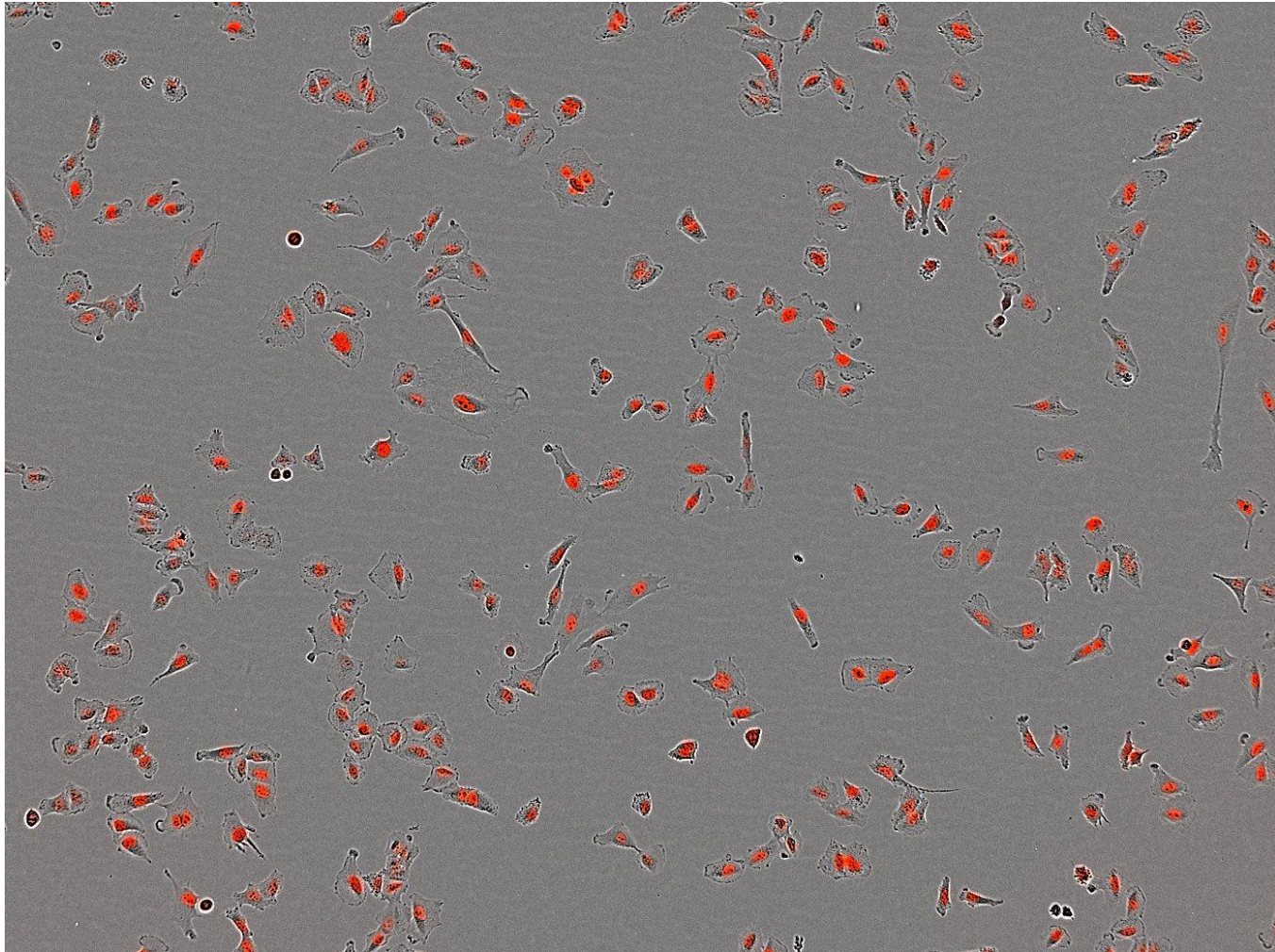
20x – 0.61 $\mu\text{m}/\text{pixel}$



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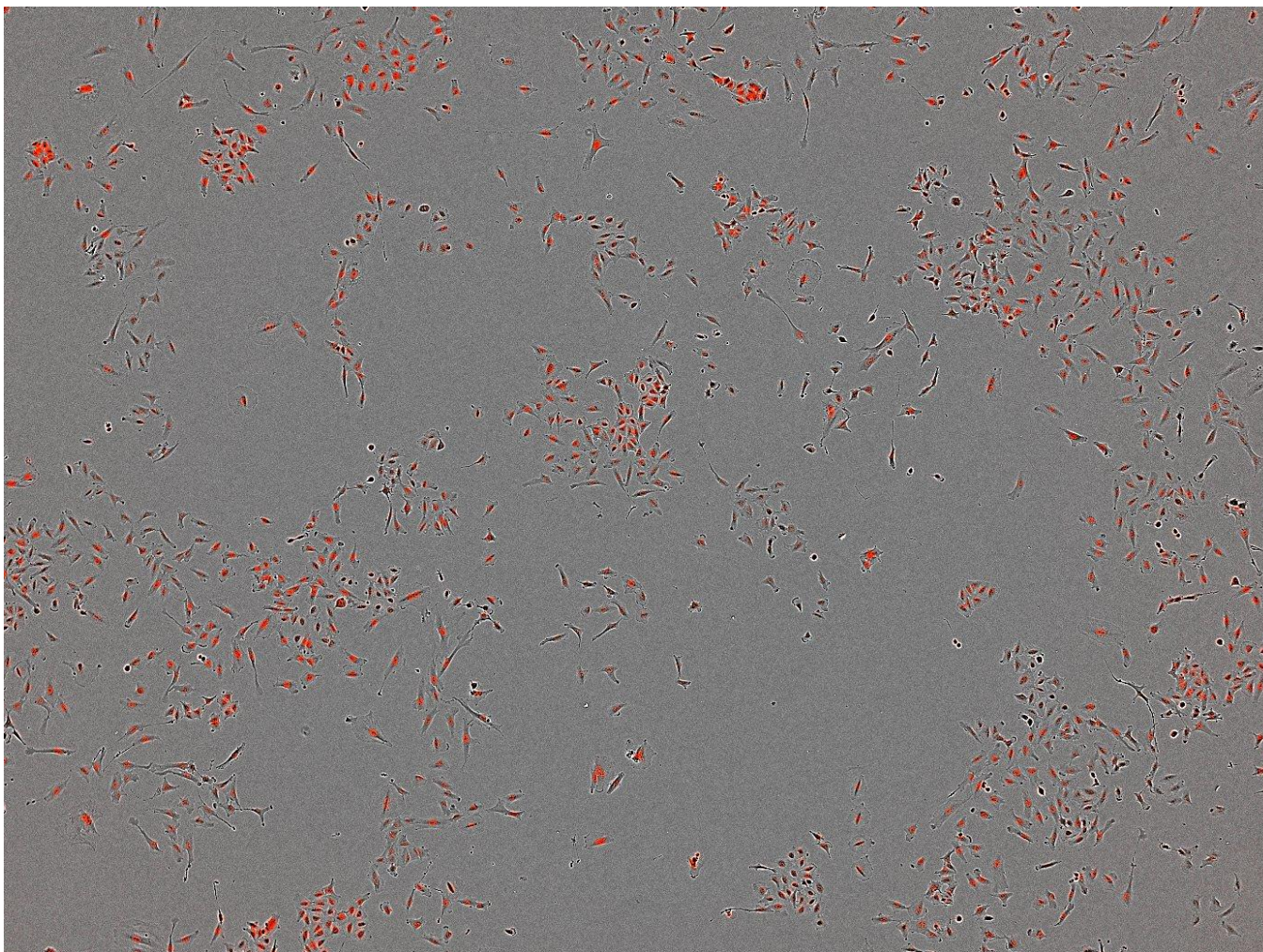
10x – 1.22 $\mu\text{m}/\text{pixel}$



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4x – 3.05 $\mu\text{m}/\text{pixel}$



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Scan Times: Standard (With Lock)

(96-well Plate; Aq time = 400 ms Green, 800 ms Red)

Objective	Channel(s)	1 per well	2 per well	3 per well
4x	Phase	9	-	-
	Phase + Green	10	-	-
	Phase + Red	11	-	-
	Phase + Green + Red	11	-	-
10x	Phase	8	15	21
	Phase + Green	9	17	25
	Phase + Red	10	18	26
	Phase + Green + Red	11	20	29
20x	Phase	9	16	24
	Phase + Green	10	18	27
	Phase + Red	10	20	29
	Phase + Green + Red	11	21	31

📌 Time in minutes to complete scan of a 96-well plate (Estimated)

Image Statistics: Standard (With Lock)

Objective	Sampling Area	Resolution	96 well coverage/image	Image size (pixels)
4x	3.95 mm x 2.96 mm; 11.72 mm ²	3.05 µm/pixel	36.63%	1296 x 972
10x	1.58 mm x 1.19 mm; 1.87 mm ²	1.22 µm/pixel	5.84%	1296 x 972
20x	0.76 mm x 0.57 mm; 0.43 mm ²	0.61 µm/pixel	1.354%	1248 x 936

- Standard (with lock) scan type is typically used to make movies, so higher resolution is probably preferred. If multiple images per well are desired, there is not much difference between 10x and 20x.
- Essen Recommends: 20x for making movies.
- Image sizes in Standard (with lock) scan type are slightly smaller than Standard as a result of image registration and cropping.

Scan Times: Scratch Wound

(96-well Plate; Aq time = 400 ms Green, 800 ms Red)

Objective	Channel(s)	1 per well	2 per well	3 per well
4x	Phase	10	-	-
	Phase + Green	11	-	-
	Phase + Red	12	-	-
	Phase + Green + Red	13	-	-
10x	Phase	9	16	-
	Phase + Green	10	18	-
	Phase + Red	11	19	-
	Phase + Green + Red	12	21	-
10x (Wide Mode)	Phase	13	25	-
	Phase + Green	16	31	-
	Phase + Red	18	35	-
	Phase + Green + Red	21	40	-
20x (Wide Mode)	Phase	26	51	76
	Phase + Green	33	64	95
	Phase + Red	37	72	107
	Phase + Green + Red	41	80	120

Time in minutes to complete scan of a 96-well plate (Estimated)

Image Statistics: Scratch Wound

(96-well Plate; Aq time = 400 ms Green, 800 ms Red)

Objective	Sampling Area	Resolution	Wound Sampling	Image size (pixels)
4x	1.92 mm x 2.31 mm; 4.43 mm ²	3.05 µm/pixel	≈1.9 mm	1296 x 972
10x	1.58 mm x 1.19 mm; 1.87 mm ²	1.22 µm/pixel	≈1.5 mm	1296 x 972
10x (Wide Mode)	1.43 mm x 1.98 mm; 2.84 mm ²	1.22 µm/pixel	≈1.4 mm	1176 x 1620
20x (Wide Mode)	0.68 mm x 1.87 mm; 1.28 mm ²	0.61 µm/pixel	≈0.65 mm	1120 x 3072

- When possible, use the 4x objective for slightly faster scan times and greater sampling

Example: 4x, 1 image per well, 1.9 mm wound sampled = 11 minutes / plate

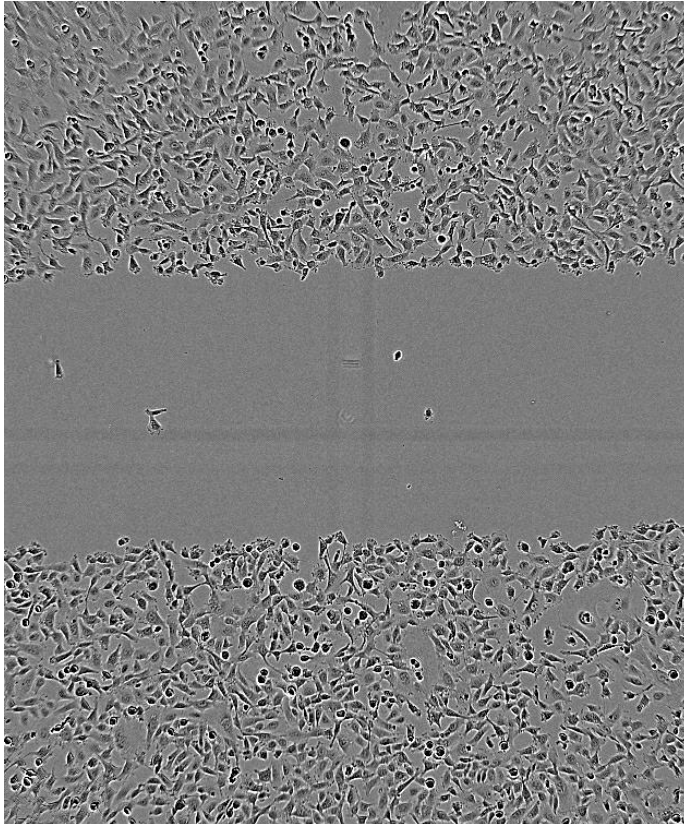
10x, (Wide Mode), Phase, 1 image per well, 1.5 mm wound sampled = 13 minutes/plate

- Essen Recommends:

- For shorter scan times and better sampling: 4x, 1 image per well
- For better resolution and sufficient sampling: 10x (Wide Mode), 1 image per well

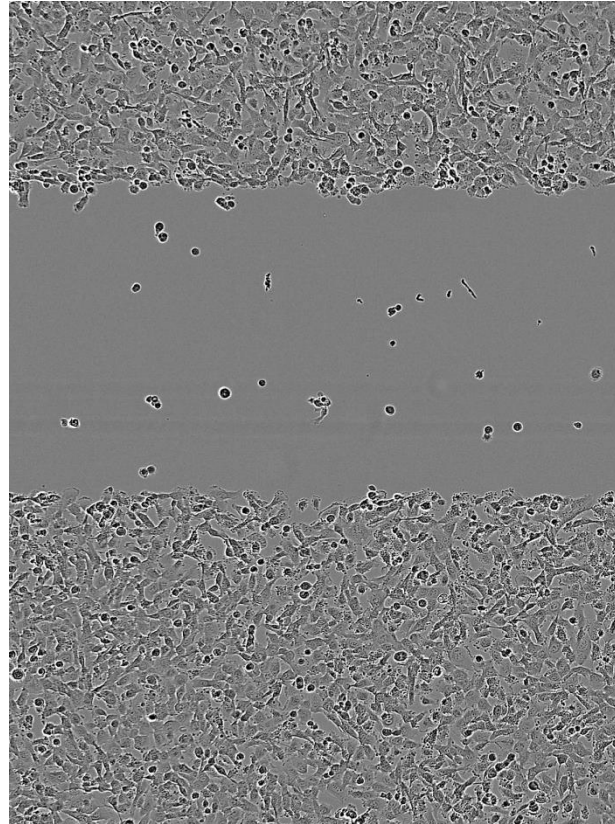
Sample Images (Scratch Wound)

4x



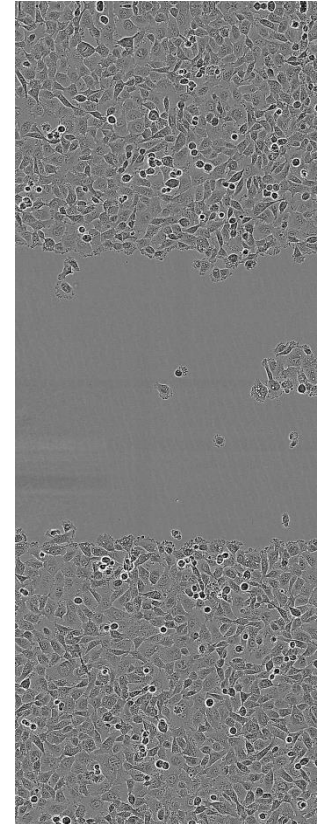
Wound Coverage= 1.9 mm

10x (Wide Mode)



1.4 mm

20x



0.65 mm

Scan Times and Image Statistics: Angiogenesis (4x vs. 10x Tiled FOV)

Objective	Channel(s)	1 per well
4x	Phase + Green	4
	Phase + Red	5
	Phase + Green + Red	6
10x (Tiled FOV)	Phase + Green	35
	Phase + Red	43
	Phase + Green + Red	52

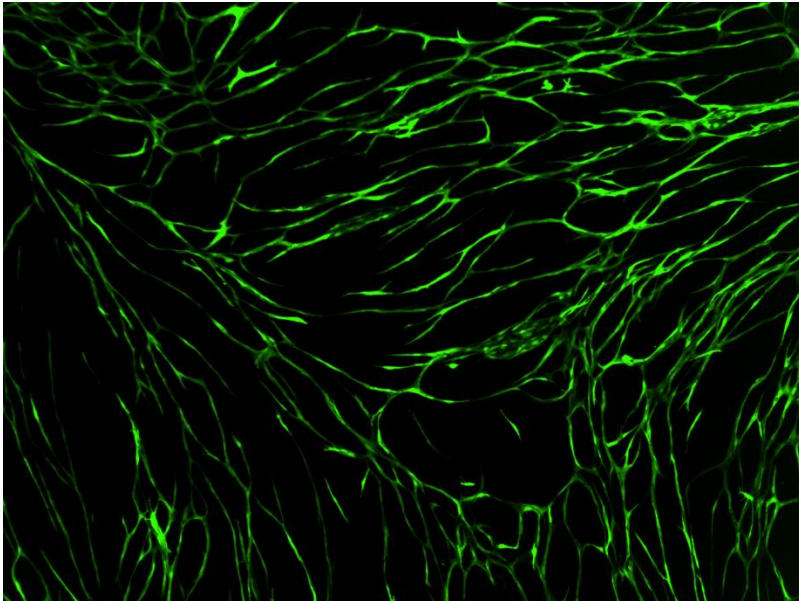
- Time in minutes to complete scan of a 96-well plate (Estimated)
- Acquisition Time = 400 ms for Green channel, 800 ms for Red channel

Objective	Sampling Area	Resolution	96 well coverage/image	Image size (pixels)
4x	4.25 mm x 3.17 mm; 13.46 mm ²	3.05 µm/pixel	42.06%	1382 x 1040
10x (Tiled FOV)	4.57 mm x 3.16 mm; 14.4 mm ²	2.44 µm/pixel	45.00%	1872 x 1296

- When possible, use the 4x objective for significant time savings. Image resolution and sampling are very similar.

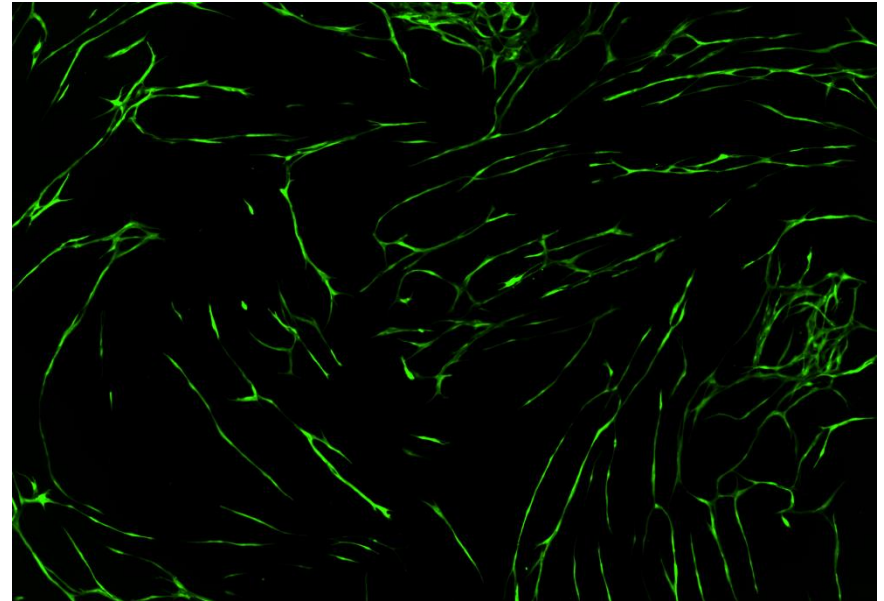
Sample Images (Angiogenesis)

4x



Area = 13.46 mm² , 42% Well Coverage

10x (Tiled FOV)



Area = 14.4 mm² , 45% Well Coverage

Scan Times: Whole Well & Dilution Cloning

(4x objective only)

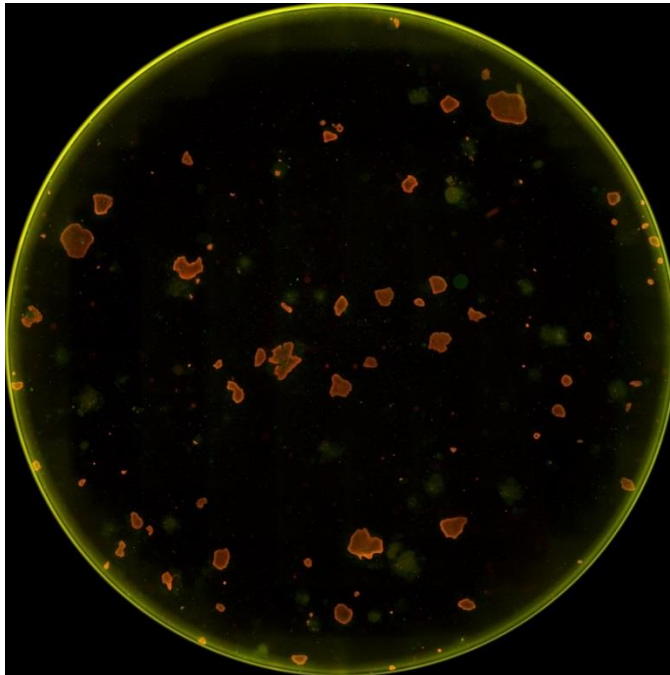
Scan Type	Channel(s)	1 per well
Whole Well	Phase	20
	Phase + Green	26
	Phase + Red	30
	Phase + Green + Red	34
Dilution Cloning	Phase	36
	Phase + Green	43
	Phase + Red	48
	Phase + Green + Red	52

- Time in minutes to complete scan of a 96-well plate (Estimated)
- Acquisition Time = 400 ms for Green channel, 800 ms for Red channel

Sample Images

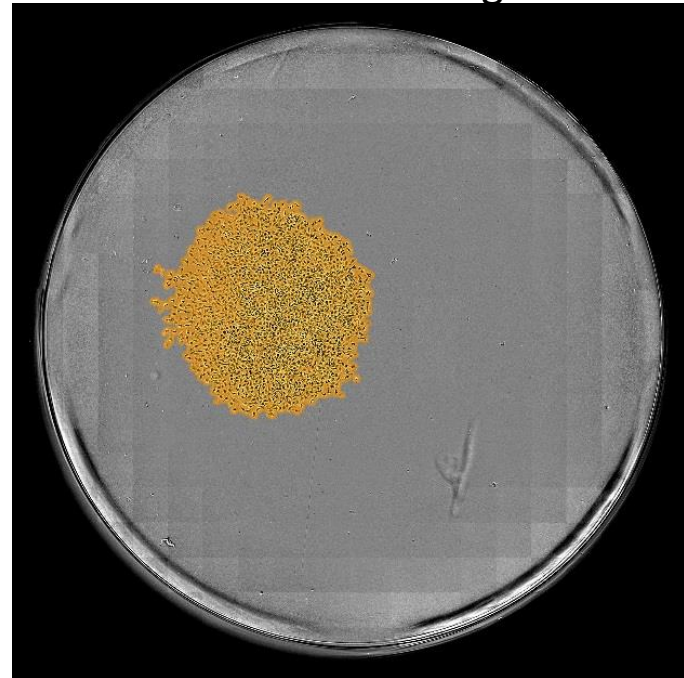
(Whole Well & Dilution Cloning)

Whole Well



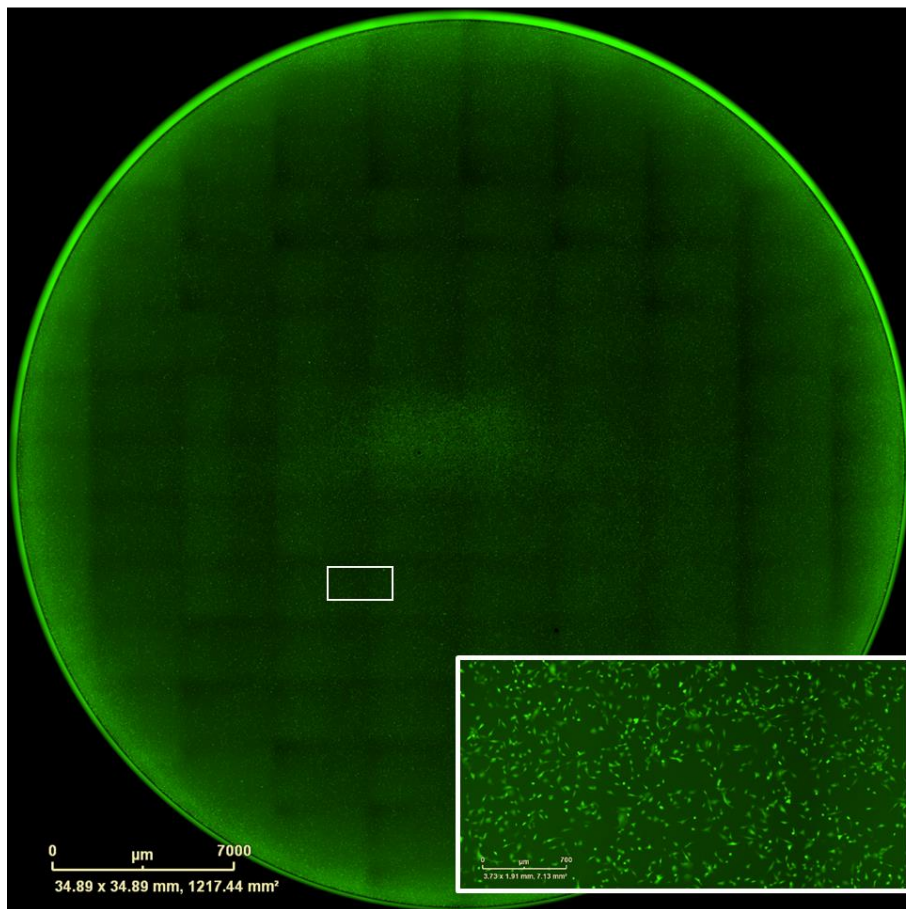
iPSC on Geltrex (feeder free)
SSEA4 – AF488 + OCT4 –AF594

Dilution Cloning

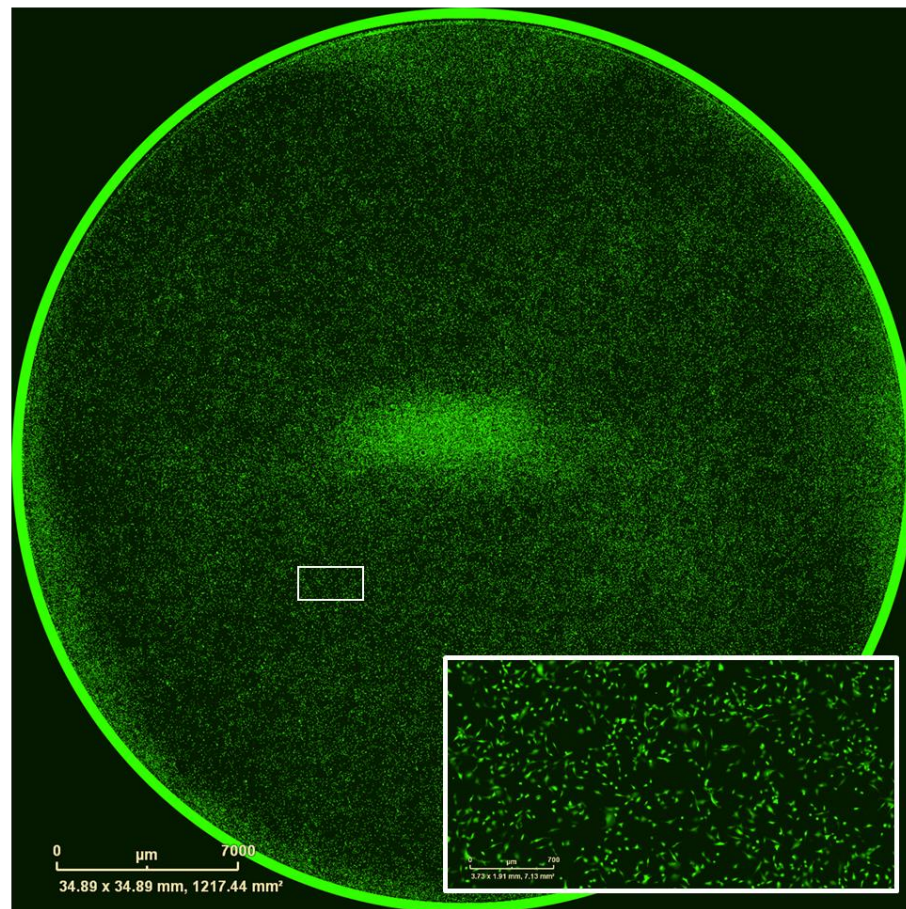


Colony identification using
HD-phase processor

Original Image



Background Subtracted: Top-Hat



Scan Times: 384-well Whole Well & Dilution Cloning

(4x objective only)

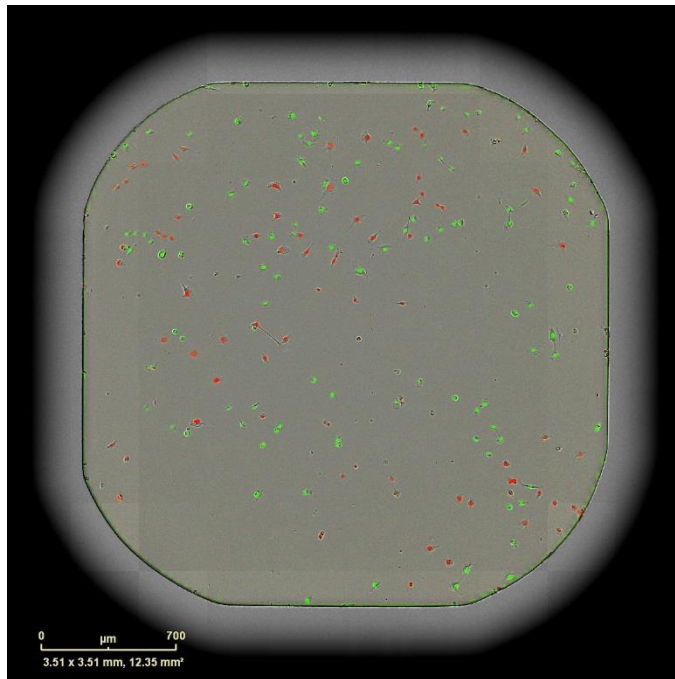
Scan Type	Channel(s)	1 per well
Whole Well	Phase	13
	Phase + Green	18
	Phase + Red	20
	Phase + Green + Red	23
Dilution Cloning	Phase	15
	Phase + Green	20
	Phase + Red	22
	Phase + Green + Red	25

- Time in minutes to complete scan of a 384-well plate (Estimated)
- Acquisition Time = 400 ms for Green channel, 800 ms for Red channel

Sample Images

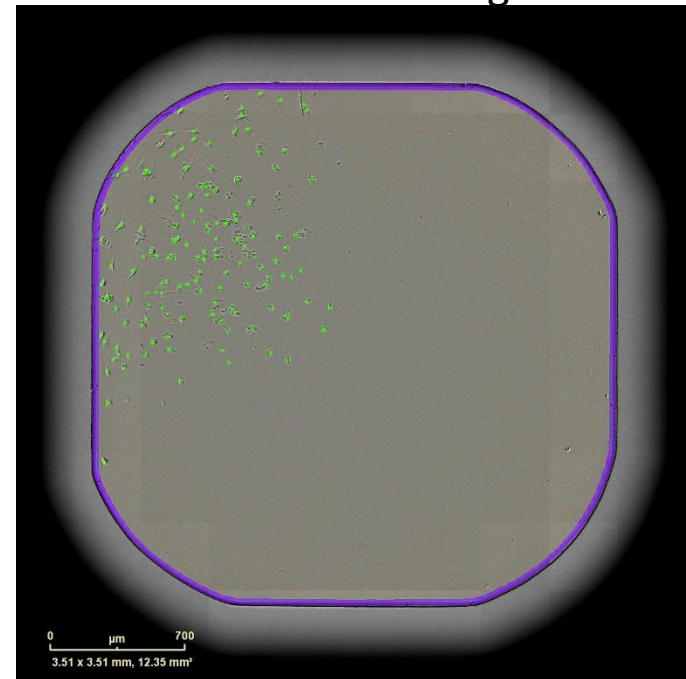
(384-well Whole Well & Dilution Cloning)

Whole Well



HT1080 NucLight Red and Green

Dilution Cloning



Colony formed from single
cell starting point

Scan Times: Chemotaxis

(10x objective only)

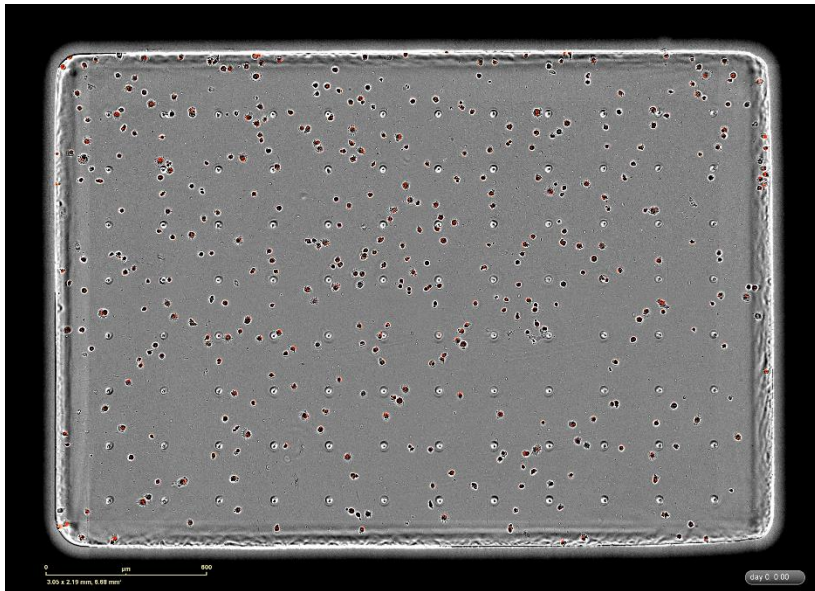
Scan Type	Channel(s)	1 per well
Chemotaxis (Top/Bot)	Phase	20
	Phase + Green	28
	Phase + Red	39
	Phase + Green + Red	23

- Time in minutes to complete scan of a 96-well ClearView Cell Migration Plate (Estimated)
- Acquisition Time = 400 ms for Green channel, 800 ms for Red channel

Sample Images

Chemotaxis (Top/Bot)

NucLight Red HT 1080



Primary Neutrophils

