Automated counting of spores in arbuscular mycorrhizal fungi *in vitro* cultures

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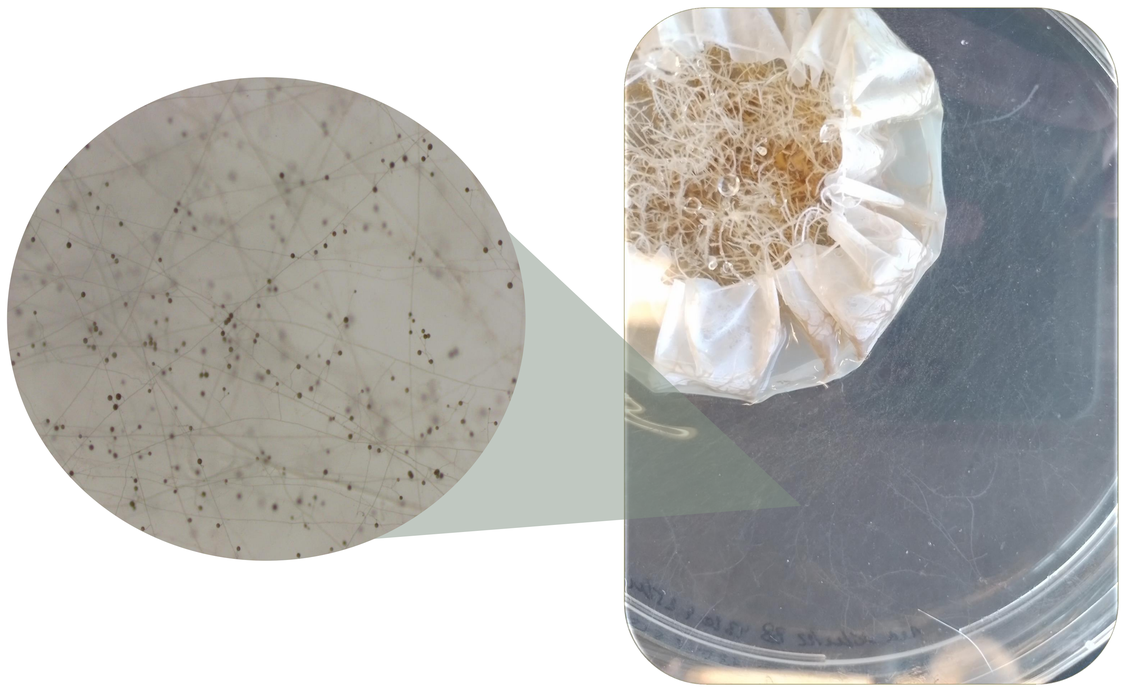
Context

- Quantification of the abundance of individuals is a key aspect of studying populations or communities
- Improvements in computer and photographic technologies

→ Possibilities to use image analysis to identify, count and characterize organisms, with several clear advantages over manual counting

Use the automatized image to replace time-consuming manual counting of spores of arbuscular mycorrhizal fungi (AMF) cultured in vitro

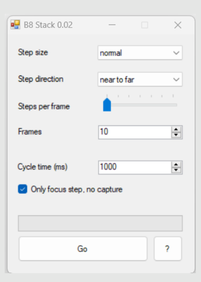


Materials & methods

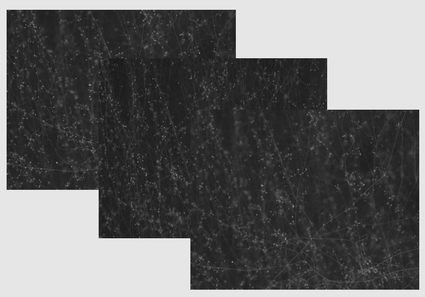
Image acquisition

Macro objective

Dark field imaging



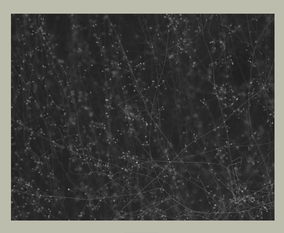
*Bracketing**



Parameters optimisation :

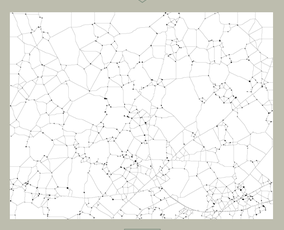
- ISO, Focus, Shutter speed
- Numbers of photos

Image analysis



Tresholding & size optimisation

Watershed optimisation



Particles analyse

Data cleaning

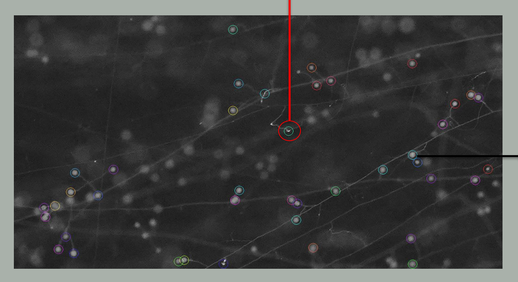
Parameters optimisation :

- Trajectories

It is based on the principle that a spore will appear on a continuous sequence of photos, unlike artifacts. Then, via a reconstruction of virtual trajectories (based on the change in depth), it is possible to discriminate spores from artifacts

Overlays of particles analyses & trajectories

Artifact



Spore

* Bracketing consists in taking several photos of the same subject by modifying camera settings. In the case presented here, the focus varies which allows to take pictures at different depths

Results

Trajectory	Frame	x	y	Area	Perimeter	Major	Minor	AR	Mean	x_corrected	y_corrected		
1	1	8736.597	21.403	1116	209.22	43.284	32.826	1.318	112.774	8736	21		
1	2	8735.559	22.118	1224	176.736	46.368	33.612	1.38	111.706	8737	20		
1	3	8733.986	23.729	1260	183.762	51.57	31.11	1.658	113.114	8737	21		
1	4	8733.405	23.571	1512	195.762	54.6	35.262	1.548	109.952	8739	19		
1	5	8733.068	24.136	1584	163.278	51.564	39.114	1.318	111.432	8741	18		
1	6	8731.866	25.524	1476	219.762	49.56	37.92	1.307	114.537	8741	18		
1	7	8729.386	27.045	1584	207.762	48.108	41.922	1.148	111.977	8741	19		
1	9	8725.459	29.969	1764	158.31	50.436	44.532	1.133	116.735	8741	19		
1	11	8722.72	31.28	1800	165.336	57.9	39.582	1.463	117.1	8742	18		
1	12	8721.233	32.411	1620	241.704	49.62	41.568	1.194	117.178	8743	17		
1	13	8718.396	35.104	1728	236.736	51.294	42.894	1.196	115.167	8742	19		
1	14	8716.413	35.935	1656	223.278	49.758	42.378	1.174	116.913	8742	18		
1	15	8712.978	37.217	1656	212.736	49.878	42.276	1.18	116.283	8740	18		
1	16	8712.183	38.256	1476	219.762	50.736	37.038	1.37	114.098	8741	18		
1	17	8710.925	39.225	1440	187.278	50.004	36.666	1.364	112.575	8742	18		
1	18	8708.265	42.147	1224	216.252	47.244	32.988	1.432	113.559	8741	19		
1	19	8707.081	43.113	1116	129.336	42	33.828	1.242	113.839	8742	19		
1	21	8705.033	43.567	1080	137.826	48.426	28.392	1.706	106.633	8744	17		
1	23	8703.735	45.324	1224	161.826	44.736	34.836	1.284	107.706	8747	16		
1	24	8700.7	47.233	1080	159.762	41.478	33.156	1.251	107.167	8746	17		
2	1	9424.1	38.217	2160	188.736	58.05	47.376	1.225	112.617	9424	38		
2	2	9423.975	39.9	1440	204.252	50.892	36.024	1.413	111.925	9426	38		
2	3	9420.91	40.577	1404	217.704	52.404	34.11	1.536	110.846	9425	37		
2	4	9420.705	40.591	1584	197.22	48.162	41.874	1.15	110.341	9427	36		
2	5	9420.223	40.883	1692	180.252	53.472	40.29	1.327	108.787	9429	35		
2	6	9419.472	41.417	1296	197.22	46.272	35.664	1.297	107.917	9431	34		
2	7	9415.833	43.167	1188	171.762	41.088	36.816	1.116	106	9429	35		
3	1	8514.155	42.864	1980	180.252	54.162	46.548	1.164	116.582	8514	42		
3	2	8513.336	43.991	1980	178.794	51.876	48.594	1.068	116.636	8515	42		
3	3	8511.582	45.643	1764	161.826	54.72	41.046	1.333	115.469	8515	43		
3	4	8511.54	45.28	1800	187.278	50.418	45.456	1.109	114.62	8517	41		
3	5	8510.761	45.652	1656	173.826	51.96	40.578	1.28	114.174	8518	40		
3	6	8509.881	47.333	1512	175.278	49.818	38.646	1.289	113	8519	40		
3	8	8505.647	49.294	1224	192.252	46.968	33.18	1.416	106.059	8518	40		

Summarize data

Conclusion

Some advantages

- Highly automated
- Images storage → New analyses with better or different approaches
- Additional and simultaneous analyses to obtain information on other aspects than abundance

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